10-14-11

EV899527137US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Fee pd

In re:	U.S. Patent No. 7,090,843
Inventor:	Francisco, et al.
Issue Date	August 15, 2006
For:	RECOMBINANT ANTI-CD30 ANTIBODIES AND USES THEREOF
Assignee:	Seattle Genetics, Inc.
Date:	October 13, 2011
Attorney Docket:	0030-00101US

BLA No.: 125388

Mail Stop: Hatch-Waxman PTE
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Commissioner for Patents:

Pursuant to 35 U.S.C. §156 and 37 C.F.R. §§1.710-1.791, Applicant, Seattle Genetics, Inc., the address of which is 21823 30th Drive Southeast, Bothell, Washington 98021, represents that it is the owner and assignee of the entire interest in and United States Patent No. 7,090,843 ("the '843 patent"), granted to Joseph A. Francisco, Grant Risdon, Alan F. Wahl, and Clay Siegall on August 15, 2006, entitled "Recombinant anti-CD30 antibodies and uses thereof," by virtue of an assignment from Joseph A. Francisco, Grant Risdon, Alan F. Wahl, and Clay Siegall recorded on January 26, 2011 (attached as **Exhibit No. 1**). The '843 patent matured from United States Patent Application No. 09/724,406, filed on November 28, 2000.

The approved product that is relevant to this application is ADCETRIS™ (brentuximab vedotin) for Injection, referred to herein as "ADCETRIS" or "Approved Product," licensed under Biologics License Application ("BLA") No. 125388.[1] The Marketing Applicant for ADCETRIS is Seattle Genetics, Inc.

The following information is submitted by Seattle Genetics through its duly authorized attorney, on behalf of Applicant (Power of Attorney attached as **Exhibit No. 2**), in accordance with 35 U.S.C. §156(d) and the rules for extension of patent term issued by the U.S. PTO at 37 C.F.R. Subpart F, §§ 1.710 to 1.791 and follows the numerical format set forth in 37 C.F.R. § 1.740.

(1) A COMPLETE IDENTIFICATION OF THE APPROVED PRODUCT AS BY APPROPRIATE CHEMICAL AND GENERIC NAME, PHYSICAL STRUCTURE OR CHARACTERISTICS:

ADCETRIS is a CD30-directed antibody-drug conjugate (ADC) consisting of three components: 1) the chimeric IgG1 antibody cAC10, specific for human CD30, 2) the microtubule disrupting agent monomethyl auristatin E ("MMAE"), and 3) a protease-cleavable linker that covalently attaches MMAE to cAC10. Approximately 4 molecules of MMAE are attached to each antibody molecule (the nominal form). Each molecule of MMAE is attached via a protease-cleavable linker.

The chemical formula of the nominal form of brentuximab vedotin is $C_{6860}H_{10532}N_{1740}O_{2168}S_{40}$.

The chemical name of brentuximab vedotin is:

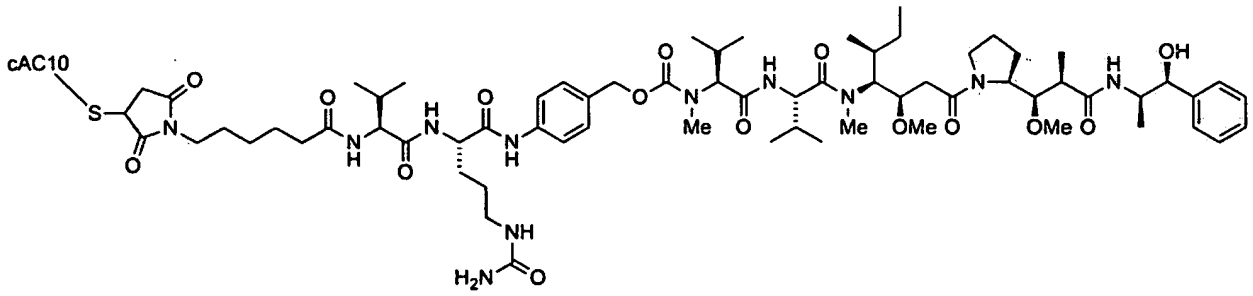
---

[1] Please note that Applicant has also applied for extension for U.S. Patent No. 7,829,531 for BLA No. 125399 pursuant to the provisions of 37 C.F.R. § 1.785.

immunoglobulin G1, anti-(human CD30 (antigen)) (human-mouse monoclonal cAC10 gamma1-chain), disulfide with human-mouse monoclonal cAC10 kappa-chain, dimer, complex with N-[[[4-[[N-[6-(2,5-dihydro-2, 5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N5-(aminocarbonyl)-L-ornithyl]amino]phenyl]methoxy]carbonyl]-N-methyl-L-valyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[[(1R,2S)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl]-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide

As noted above, an average of four (4) protease cleavable linker-MMAE molecules are attached to some of the interchain disulfides of the antibody.

Brentuximab vedotin has the following chemical structure (as presented in the USPI):



The nominal form has an average of four protease cleavable linker-MMAE molecules per antibody.

(2) A COMPLETE IDENTIFICATION OF THE FEDERAL STATUTE INCLUDING THE APPLICABLE PROVISION OF LAW UNDER WHICH THE REGULATORY REVIEW OCCURRED:

The Approved Product is a product the regulatory review period for which occurred under both the Federal Food, Drug, and Cosmetic Act ("FDC Act") (21 U.S.C. § 355(i)) and the Public Health Service Act ("PHS Act") (42 U.S.C. § 262(a)).

(3) AN IDENTIFICATION OF THE DATE ON WHICH THE PRODUCT RECEIVED PERMISSION FOR COMMERCIAL MARKETING OR USE UNDER THE PROVISION OF LAW UNDER WHICH THE APPLICABLE REGULATORY REVIEW PERIOD OCCURRED:

The Approved Product received permission for commercial marketing or use by the Food and Drug Administration ("FDA") pursuant to PHS Act § 351(a) in a letter dated August 19, 2011. A copy of the approval letter is attached as **Exhibit No. 3**.

(4) IN THE CASE OF A DRUG PRODUCT, AN IDENTIFICATION OF EACH ACTIVE INGREDIENT IN THE PRODUCT AND AS TO EACH ACTIVE INGREDIENT, A STATEMENT THAT IT HAS NOT BEEN PREVIOUSLY APPROVED FOR COMMERCIAL MARKETING OR USE UNDER THE FFDCA, THE PUBLIC HEALTH SERVICE ACT, OR THE VIRUS-SERUM-TOXIN ACT OR A STATEMENT OF WHEN THE ACTIVE INGREDIENT WAS APPROVED FOR COMMERCIAL MARKETING OR USE (EITHER ALONE OR IN COMBINATION WITH OTHER ACTIVE INGREDIENTS), THE USE FOR WHICH IT WAS APPROVED AND THE PROVISION OF LAW UNDER WHICH IT WAS APPROVED: (37 C.F.R. § 1.740(a)(4))

ADCETRIS has been licensed under PHS Act § 351(a) for: (1) the treatment of patients with Hodgkin lymphoma after failure of autologous stem cell transplant ("ASCT") or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates (BL 125388) ; and (2) the treatment of patients with systemic anaplastic large cell lymphoma after failure of at least one prior multi-agent chemotherapy regimen (BL 125399) (collectively described in the Approved Label (US Prescribing Information) attached as **Exhibit No. 4**). The Approved Product, brentuximab vedotin, is an ADC comprising an anti-CD30 monoclonal antibody attached by protease-cleavable linkers to the microtubule disrupting agent, MMAE.

Neither brentuximab vedotin, nor either of its drug and biologic components, nor any salt or ester of a component of brentuximab vedotin, have been previously approved for commercial marketing or use under the FDC Act, the PHS Act, or the Virus-Serum-Toxin Act.

(5) A STATEMENT THAT THE APPLICATION IS BEING SUBMITTED WITHIN THE 60 DAY PERIOD PERMITTED FOR SUBMISSION PURSUANT TO SECTION 1.720(f) AND AN IDENTIFICATION OF THE DATE OF THE LAST DAY ON WHICH THE APPLICATION COULD BE SUBMITTED.

This Application is timely filed, pursuant to 35 U.S.C. §156(d)(1), within the permitted sixty (60) day period that began on August 19, 2011, when the product received permission under 42 U.S.C § 351(a) and that will expire on October 18, 2011.

(6) A COMPLETE IDENTIFICATION OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT BY THE NAME OF THE INVENTOR, THE PATENT NUMBER, THE DATE OF ISSUE AND THE DATE OF EXPIRATION:

U.S. Patent No.	7,090,843
Inventors:	Francisco, *et al.*
Date of Issue:	August 15, 2006
Expiration Date:	November 28, 2020 (without extension under 35 U.S.C. § 156)

(7) A COPY OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT, INCLUDING THE ENTIRE SPECIFICATION (INCLUDING CLAIMS) AND DRAWINGS:

A complete copy of the '843 patent is attached as **Exhibit No. 5**.

(8) A COPY OF ANY DISCLAIMER, CERTIFICATE OF CORRECTION, RECEIPT OF MAINTENANCE FEE PAYMENT, OR RE-EXAMINATION CERTIFICATE ISSUED IN THE U.S. PATENT:

The '843 patent is not subject to any disclaimer.

The '843 patent has not been re-examined, and so no re-examination certificate has been issued.

No Certificate of Correction has issued for the '843 patent.

The fourth year maintenance fee for the '843 patent was paid on February 16, 2010 (receipt attached as **Exhibit No. 6**). There are no unpaid maintenance fees for this patent.

(9) A STATEMENT THAT THE PATENT CLAIMS THE APPROVED PRODUCT, OR A METHOD OF USING OR MANUFACTURING THE APPROVED PRODUCT, AND A SHOWING WHICH LISTS EACH APPLICABLE PATENT CLAIM AND DEMONSTRATES THE MANNER IN WHICH AT LEAST ONE SUCH PATENT CLAIM READS ON THE APPROVED PRODUCT OR A METHOD OF USING OR MANUFACTURING THE APPROVED PRODUCT:

The '843 patent claims the Approved Product. Specifically, claims 1, 2, 4, 7, 8, 9, 10, 12, 16, 17 and 18 read on the Approved Product. Pursuant to 37 C.F.R. §1.740(a)(9), a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on the approved product is set forth in the table below.

CLAIM	ELEMENTS
1. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject, in an amount effective for said treatment, (a) an antibody that (i) immunospecifically binds CD30 and (ii) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, wherein said antibody exerts the cytostatic or cytotoxic effect on the Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent and in the absence of cells other than cells of said Hodgkin's Disease cell line; and (b) a pharmaceutically acceptable carrier.	The Approved Product received permission for treating certain patients with Hodgkin lymphoma. Hodgkin lymphoma is also referred to as Hodgkin's Disease. The antibody is chimeric AC10 antibody (cAC10). cAC10 immunospecifically binds to human CD30 protein. The cAC10 antibody can exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of other cells and in the absence of conjugation to a cytostatic or cytotoxic agent. The Approved Product contains a pharmaceutically acceptable carrier, which is an excipient, such as trehalose, sodium citrate, citric acid and/or polysorbate 80.
2. The method of claim 1, wherein the antibody is human, humanized or chimeric.	The antibody in the Approved Product, cAC10, is a chimeric antibody.
4. The method of claim 1, wherein the antibody is conjugated to a cytotoxic agent.	The Approved Product contains a cytotoxic agent, MMAE (a microtubule-disrupting agent), that is conjugated to the antibody, cAC10.

CLAIM	ELEMENTS
7. The method of claim 1, wherein the cytostatic or cytotoxic effect of the antibody is exhibited upon performing a method comprising: (a) contacting a culture of the Hodgkin's Disease cell line with the antibody, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours; (b) exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72 hour period; and (c) measuring the incorporation of the ^{3}H-thymidine into cells of the culture, wherein the antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the antibody.	The antibody in the Approved Product, cAC10, exhibits a cytostatic or cytotoxic effect in the recited method.
8. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject an amount of an antibody, which antibody (a) competes for binding to CD30 with monoclonal antibody AC10 or HeFi-1, and (b) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of cell other than cells of said Hodgkin's Disease cell line, which amount is effective for the treatment of Hodgkin's Disease.	The Approved Product received permission for treating certain patients with Hodgkin lymphoma. Hodgkin lymphoma is also referred to as Hodgkin's Disease. The antibody is chimeric AC10 antibody (cAC10), which competes with AC10 antibody for binding to human CD30 protein. The cAC10 antibody can exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of other cells. The Approved Product is active on Hodgkin's Disease.

CLAIM	ELEMENTS
9. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject an amount of an antibody, which antibody (a) comprises the amino acid sequence that has at last 95% identity to SEQ ID NO:2, (b) immunospecifically binds CD30, and (c) exerts a cytostatic or cytostatic effect on a Hodgkin's Disease cell line in the absence of cells other than cells of said Hodgkin's Disease cell line which amount is effective for the treatment of Hodgkin's Disease.	The Approved Product received permission for treating certain patients with Hodgkin lymphoma. Hodgkin lymphoma is also referred to as Hodgkin's Disease. The cAC10 antibody in the Approved Product has a variable region having the amino acid sequence as set forth of SEQ ID NO:2. The cAC10 antibody immunospecifically binds to human CD30 protein. The cAC10 antibody can exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of other cells. The Approved Product is active on Hodgkin's Disease.
10. The method of any one of claims 8 or 9, wherein the antibody is a human, humanized or chimeric antibody.	The antibody in the Approved Product, cAC10, is a chimeric antibody.
12. The method of any one of claims 8 or 9, wherein the antibody is conjugated to a cytotoxic agent.	The Approved Product contains a cytotoxic agent, MMAE (a microtubule-disrupting agent) that is conjugated to the antibody, cAC10.

CLAIM	ELEMENTS
16. The method of any one of claims 8 or 9, wherein the cytostatic or cytotoxic effect is exhibited upon performing a method comprising: (a) contacting a culture of the Hodgkin's Disease cell line with the antibody, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours; (b) exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72 hour period; and (c) measuring the incorporation of the ^{3}H-thymidine into cells of the culture, wherein the antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the antibody.	The antibody in the Approved Product, cAC10, exhibits a cytostatic or cytotoxic effect in the recited method.
17. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject, in an amount effective for said treatment, (a) an antibody that (i) immunospecifically binds CD30 and (ii) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, wherein the antibody exerts the cytostatic or cytotoxic effect on the Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent and	The Approved Product received permission for treating certain patients with Hodgkin lymphoma. Hodgkin lymphoma is also referred to as Hodgkin's Disease. The Approved Product is active on Hodgkin's Disease. The antibody in the Approved Product, cAC10, immunospecifically binds to human CD30 protein. The cAC10 antibody can exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent.

CLAIM	ELEMENTS
(b) a pharmaceutically acceptable carrier,	The Approved Product contains a pharmaceutically acceptable carrier, which is an excipient, such as trehalose, sodium citrate, citric acid and/or polysorbate 80.
wherein the cytostatic or cytotoxic effect of the antibody is exhibited upon performing a method comprising: (A) immobilizing said antibody in a well, said well having a culture area of about 0.33 cm^2; (B) adding about 5,000 cells of the Hodgkin's Disease cell line in the presence of RPMI with 20% fetal bovine serum to the well: (C) culturing the cells in the presence of said antibody and RPMI with 20% fetal bovine scrum for a period of 72 hours to form a Hodgkin's Disease cell culture; (D) exposing the Hodgkin's Disease cell culture to 0.5 μCi/well of ^{3}H-thymidine during the final 8 hours of said 72 hour period; and (E) measuring the incorporation of the ^{3}H-thymidine into cells of the Hodgkin's Disease cell culture, wherein the antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the Hodgkin's Disease cell culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the antibody.	The antibody in the Approved Product, cAC10, also exhibits a cytostatic or cytotoxic effect in the recited method.

CLAIM	ELEMENTS
18. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject, in an amount effective for said treatment,	The Approved Product received permission for treating certain patients with Hodgkin lymphoma. Hodgkin lymphoma is also referred to as Hodgkin's Disease. The Approved Product is active on Hodgkin's Disease.
(a) a chimeric, humanized or human antibody that (i) immunospecifically binds CD30 and (ii) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, wherein the chimeric, humanized or human antibody exerts the cytostatic or cytotoxic effect on Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent and	The antibody in the Approved Product, cAC10, immunospecifically binds to human CD30 protein. The cAC10 antibody can exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent.
(b) a pharmaceutically acceptable carrier,	The Approved Product contains a pharmaceutically acceptable carrier, which is an excipient, such as trehalose, sodium citrate, citric acid and/or polysorbate 80.
wherein the cytostatic or cytotoxic effect of the chimeric, humanized or antibody is exhibited upon performing a method comprising: (A) contacting a culture of the Hodgkin's Disease cell line with the chimeric, humanized or human antibody, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours; (B) adding a cross-linking antibody to the Hodgkin's Disease cell line, the cross-linking antibody binding to the chimeric, humanized or human antibody; (C) exposing the culture to 0.5 μCi of ^3H-thymidine during the final 8 hours of said	The antibody in the Approved Product, cAC10, also exhibits a cytostatic or cytotoxic effect in the recited method.

CLAIM	ELEMENTS
72-hour period; and (D) measuring the incorporation of the ^{3}H-thymidine into cells of the culture, wherein the chimeric, humanized or human antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the chimeric, humanized or human antibody.	

(10) A STATEMENT BEGINNING ON A NEW PAGE OF THE RELEVANT DATES AND INFORMATION PURSUANT TO 35 U.S.C. §156(g) IN ORDER TO ENABLE THE SECRETARY OF HEALTH AND HUMAN SERVICES OR THE SECRETARY OF AGRICULTURE, AS APPROPRIATE, TO DETERMINE THE APPLICABLE REGULATORY REVIEW PERIOD AS FOLLOWS:

(i) FOR A PATENT CLAIMING A HUMAN DRUG, ANTIBIOTIC OR HUMAN BIOLOGICAL PRODUCT, THE EFFECTIVE DATE OF THE INVESTIGATIONAL NEW DRUG APPLICATION (IND) AND THE IND NUMBER; THE DATE ON WHICH A NEW DRUG APPLICATION (NDA) OR A PRODUCT LICENSE APPLICATION (PLA) WAS INITIALLY SUBMITTED AND THE NDA OR PLA NUMBER; AND THE DATE ON WHICH THE NDA WAS APPROVED OR THE PRODUCT LICENSE ISSUED:

An original Investigational New Drug Application ("IND") was submitted on June 28, 2006 and assigned IND No. 71,634. A copy of the letter acknowledging receipt of the IND is attached as **Exhibit No. 7**. The IND became effective on July 27, 2006 (*i.e.*, 30 days from submitting the IND).

A BLA was submitted on February 25, 2011 for two proposed uses: (1) relapsed or refractory Systemic Anaplastic Large Cell Lymphoma, and (2) relapsed or refractory Hodgkin's Lymphoma. The FDA split the original BLA into two applications, BL 125388 and BL125399[2], one for each proposed use (Hodgkin lymphoma and anaplastic large cell lymphoma, respectively). Thus, each BLA was subject to separate regulatory review.

[2] Please note that Applicant has also applied for extension for U.S. Patent No. 7,829,531 for BLA No. 125399 pursuant to the provisions of 37 C.F.R. § 1.785.

A letter, dated March 2, 2011, acknowledged receipt by FDA of the BLA No. 125388 for relapsed or refractory Hodgkin's Lymphoma on February 28, 2011 (attached as **Exhibit No. 8**). Accordingly, the BLA that is the subject of the instant application (No. 125388) was initially submitted on February 28, 2011. BLA No. 125388 was licensed (*i.e.*, approved) on August 19, 2011.

(11) A BRIEF DESCRIPTION BEGINNING ON A NEW PAGE OF THE SIGNIFICANT ACTIVITIES UNDERTAKEN BY THE MARKETING APPLICANT, DURING THE APPLICABLE REGULATORY REVIEW PERIOD WITH RESPECT TO THE APPROVED PRODUCT AND THE SIGNIFICANT DATES APPLICABLE TO SUCH ACTIVITIES:

In accordance with 37 C.F.R. §1.740(a)(11), a list of significant activities undertaken by the Marketing Applicant, its predecessors, and affiliates, in IND No. 71,634 and BLA No. 125388 during the applicable regulatory review period with respect to the Approved Product is provided below. Applicant lists significant activities relevant to clinical trials for both proposed uses because the data from these trials supports the BLA.

SUBMISSION DATE	SERIAL NUMBER	DESCRIPTION
6/28/2006	000	IND Submitted to FDA - Phase I Open-Label Dose Finding Study of SGN-35 for CD30 Positive Hematologic Malignancies (NCT00430846) (SG035-0001)
7/27/2006	NA	IND is cleared to proceed
12/14/2006	NA	Submitted application for orphan drug designation (Hodgkin Lymphoma)
1/30/2007	NA	Orphan drug designation granted (Hodgkin Lymphoma)
9/12/2007	0023	New Protocol - A Phase I Dose Escalation Study of SGN-35 Alone and in Combination With Gemcitabine for CD30-Positive Malignancies (NCT00649584) (SG035-0002)
12/10/2007	0030	CMC amendment – vial seals
5/22/2008	0043	SG035-0001 - End-of-Phase 1 (EOP1) meeting request
7/24/2008	NA	SG035-0001 - EOP 1 meeting
8/25/2008	0051	Information amendment – population PK report
9/10/2008	NA	Application for Orphan Drug Designation (Anaplastic Large Cell Lymphoma)
9/11/2008	0052	Special Protocol Assessment (SPA) request – A Pivotal Open-Label Trial of SGN-35 for Hodgkin Lymphoma (NCT00848926) (SG035-0003

SUBMISSION DATE	SERIAL NUMBER	DESCRIPTION
9/15/2008	0053	Special Protocol Assessment (SPA) request – A Phase 2 Open-Label Trial of SGN-35 for Systemic Anaplastic Large Cell Lymphoma (NCT00866047) (SG035-0004)
9/19/2008	0054	CMC meeting request
11/17/2008	NA	FDA response to CMC meeting request questions – meeting cancelled
10/23/2008	NA	Orphan drug designation granted (ALCL)
1/13/2009	NA	ALCL SPA non-agreement
1/16/2009	NA	SG035-0003 - HL SPA agreement
1/20/2009	0068	Request for Fast Track (HL)
1/22/2009	0069	Meeting request – nonclinical and clinical pharmacology
1/30/2009	0070	Request for Fast Track (ALCL)
2/12/2009	0071	New Protocol – An SGN-35 Trial for Patients Who Have Previously Participated in an SGN-35 Study (NCT00947856) (SGN35-006)
3/13/2009	NA	Fast Track non agreement – ALCL
3/13/2009	NA	SG035-0003 – Fast Track agreement – HL
4/28/2009	0080	CMC – comparability plan
4/30/2009	0081	Meeting request – A Phase 3 Study of Brentuximab Vedotin (SGN-35) in Patients at High Risk of Residual Hodgkin Lymphoma Following Stem Cell Transplant (The AETHERA Trial) SGN35-005
6/11/2009	0088	New Protocol – Cardiac Safety Study of Brentuximab Vedotin (SGN-35) (NCT01026233) (SGN35-007)
6/16/2009	NA	SGN35-005 non-agreement
7/27/2009	0093	Meeting Request – SGN35-005
8/7/2009	0095	Clinical Pharmacology update
8/31/2009	0102	New Protocol – Clinical Pharmacology Study of Brentuximab Vedotin (SGN-35) (NCT01026415) (SGN35-008)
10/19/2009	0119	New Protocol – A Phase 1 Study of Brentuximab Vedotin Combined With Multi-Agent Chemotherapy for Hodgkin Lymphoma (NCT01060904) (SGN35-009)
11/10/2009	0127	Meeting Request – CMC
1/19/2010	NA	CMC meeting
3/09/2010	0163	Clinical study report – SG035-0001
3/29/2010	0173	Request for proprietary name review
5/4/2010	0190	New Protocol – A Treatment-Option Study of Brentuximab

SUBMISSION DATE	SERIAL NUMBER	DESCRIPTION
		Vedotin in Patients With Progression of Hodgkin Lymphoma or Systemic Anaplastic Large Cell Lymphoma (NCT01196208) (SGN35-010)
5/21/2010	0207	Pre BLA planning meeting request
8/12/2010	NA	Pre BLA planning meeting
9/03/2010	0252	Request for Pre-BLA meeting – clinical
11/18/2010	NA	Pre-BLA meeting – clinical
9/16/2010	0258	Request for proprietary name review
9/20/2010	0260	Pre BLA CMC meeting request
10/5/2010	0271	New Protocol – A Phase 1 Study of Brentuximab Vedotin Given Sequentially and Combined With Multi-Agent Chemotherapy for Anaplastic Large Cell Lymphoma (NCT01309789) (SGN35-011)
11/29/2010	0297	CMC amendment
12/7/2010	NA	Pre BLA CMC meeting
2/25/2011	000	BLA No. 125388 Submission
2/28/2011	NA	BLA No. 125388 FDA Receipt
3/14/2011	NA	Conditional approval for name ADCETRIS
8/19/2011		BLA No. 125388 Approval

(12) A STATEMENT BEGINNING ON A NEW PAGE THAT IN THE OPINION OF THE APPLICANT THE PATENT IS ELIGIBLE FOR THE EXTENSION AND A STATEMENT AS TO THE LENGTH OF THE EXTENSION CLAIMED, INCLUDING HOW THE LENGTH OF EXTENSION WAS DETERMINED:

Applicant is of the opinion that the '843 patent is eligible for extension under 35 U.S.C. § 156, because it satisfies all of the requirements for such extension as follows:

a. 35 U.S.C. §156(a); 37 C.F.R. § 1.720(a)

The '843 patent claims a product, and methods of using a product.

b. 35 U.S.C. § 156(a)(l); 37 C.F.R. § 1.720(g)

The term of the '843 patent has not expired before submission of this application.

c. 35 U.S.C. § 156(a)(2); 37 C.F.R. § 1.720(b)

The term of the '843 patent has never previously been extended under 35 U.S.C. § 156.

d. 35 US.C. § 156(a)(3); 37 C.F.R. § 1.730

This application for extension is submitted by the authorized agent or the owner of record in accordance with the requirement of 35 U.S.C. § 156(d) and the rules of the U.S. PTO.

e. 35 U.S.C. §156(a)(4); 37 C.F.R. § 1.720(d)

The product, ADCETRIS (brentuximab vedotin) for Injection, has been subject to a regulatory review period as defined in 35 U.S.C. § 156(g) before its commercial marketing or use.

f. 35 U.S.C. §156(a)(5)(A); 37 C.F.R. § 1.720(e)(i)

The commercial marketing or use of the Approved Product after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the PHS Act under which such regulatory review period occurred.

g. 35 U.S.C. § 156(c)(4); 37 C.F.R. § 1.720(h)

No other patent has been extended for the same regulatory review period for the Approved Product.

h. 35 U.S.C. § 156(d)(l); 37 C.F.R. § 1.720(f)

This application is submitted within the permitted 60 day period beginning on the date the product first received permission for commercial marketing or use.

Applicant is of the opinion that the term of the '843 patent, currently expiring on November 28, 2020, is eligible for extension under 35 U.S.C. § 156 for ***1002 days***, to August 27, 2023, as determined pursuant to 37 C.F.R. § 1.775 as follows:

Patent Information:

Patent Issue Date – August 15, 2006

Current Patent Expiration Date (20-year term) – November 28, 2020

FDA Information:

Date IND Became Effective – July 27, 2006

Date NDA Submitted to the FDA – February 28, 2011

Date NDA Approved by the FDA – August 19, 2011

IND Period:

Start Date of Regulatory Review Period – July 27, 2006

IND Period (days) – 1678

½ IND Period (days) – 839

Regulatory Review Period Allowed:

NDA Review Period (days) – 173

Regulatory Review Period (days) – 1851

Reg. Rev. Period less ½ IND period (days) – 1012

Reg. Rev. Period less Days Before Patent Grant (days) – 1002

Statutory Limitations:

Expiration under 5 year extension limitation (Date 1) – Not Applicable

Expiration under 14 years from NDA approval limitation (Date 2) – August 19, 2025

Expiration based upon full regulatory review period (Date 3) – August 27, 2023

Final Expiration Date (Earliest of Date 1, Date 2, or Date 3) – August 27, 2023

Maximum Extension in Days: 1002 Days

(13) A STATEMENT THAT APPLICANT ACKNOWLEDGES A DUTY TO DISCLOSE TO THE DIRECTOR OF THE UNITED STATES PATENTS AND TRADEMARK OFFICE AND THE SECRETARY OF HEALTH AND HUMAN SERVICES ANY INFORMATION WHICH IS MATERIAL TO THE DETERMINATION OF ENTITLEMENT TO THE EXTENSION SOUGHT (SEE 37 C.F.R. §1.765):

The Applicant, Seattle Genetics, through the undersigned attorney, acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) THE PRESCRIBED FEE FOR RECEIVING AND ACTING UPON THE APPLICATION FOR EXTENSION (SEE 37 C.F.R. § 1.20(j)):

The Director is hereby authorized to charge a fee of $1120.00 to cover the fee for a request for extension of patent term to Deposit Account No. 50-2900, and any deficiency in the fees filed, asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm), to prevent this application from being inadvertently abandoned.

(15) THE NAME, ADDRESS AND TELEPHONE NUMBER OF THE PERSON TO WHOM INQUIRIES AND CORRESPONDENCE RELATING TO THE APPLICATION FOR PATENT TERM EXTENSION ARE TO BE DIRECTED:

Mark Sandbaken
Seattle Genetics, Inc.
21823 30th Drive SE
Bothell, WA 98021

(425) 527-4138 (direct)

(425) 527-4109 (fax)

Pursuant to 37 C.F.R. § 1.740(b), this Request for Extension of Patent Term under 35 U.S.C. § 156, including exhibits, is being filed in triplicate.

(16) AN OATH OR DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: October 13, 2011

Respectfully submitted,

By 
Mark G. Sandbaken
Reg. No. 39,354

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APRIL 26, 2001

PTAS

101615353A

Antler
24273

PENNIE & EDMONDS LLP
ADRIANE M. ANTLER
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 01/26/2001

REEL/FRAME: 011508/0339
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
FRANCISCO, JOSEPH A.

DOC DATE: 01/10/2001

ASSIGNOR:
RISDON, GRANT

DOC DATE: 01/09/2001

ASSIGNOR:
WAHL, ALAN F.

DOC DATE: 01/10/2001

ASSIGNOR:
SIEGALL, CLAY

DOC DATE: 01/10/2001

ASSIGNEE:
SEATTLE GENETICS, INC.
22215 26TH AVENUE, S.E.
BOTHELL, WASHINGTON 98021

SERIAL NUMBER: 09724406
PATENT NUMBER:

FILING DATE: 11/28/2000
ISSUE DATE:

REFERRED TO
REC'D
MAY 04 2001
Pennie & Edmonds
O.K. for filing

JEFFREY OLSEN, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

01-29-01 $D

Express Mail No. EL 501 739 383 US

OIPE JC22 JAN 26 2001 PATENT & TRADEMARK OFFICE

02-15-2001

101615353

Attorney Docket Number 9632-006-999

TO THE HONORABLE ... DEMARKS

Washington, DC 20231

1-26-01

Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

Joseph A. Francisco, Grant Risdon, Alan F. Wahl and Clay Siegall

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: Seattle Genetics, Inc.

Address: 22215 26th Avenue, S.E.
Bothell, Washington 98021

Country (if other than USA): _____

3. Nature of conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other _____

Execution Date: January 10, 2001;
January 9, 2001;
January 10, 2001; and
January 10, 2001, respectively

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: _____

A. Patent Application No.(s) 09/724,406

B. Patent No.(s) _____

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, NY 10036

6. Number of applications and patents involved: 1

7. Total fee (37 CFR 3.41):.....$ 40.00
Please charge to the deposit account listed in Section 8.

8. Deposit account number:
16-1150

01 FC:481 40.00 DP

DO NOT USE THIS SPACE

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Adriane M. Antler	32,605	Adriane M. Antler	January 26, 2001
Name of Person Signing	Reg. No.	Signature	Date

Total number of pages including cover sheet: 3

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents & Trademarks, Box Assignment
Washington, D.C. 20231

ASSIGNMENT

WHEREAS, WE, JOSEPH A. FRANCISCO, citizen of the United States, residing at at 21705 92nd Avenue West, Edmonds, Washington 98020, GRANT RISDON, citizen of the United States, residing at 7400 Northmoor, Clayton, Missouri 63105, ALAN F. WAHL, citizen of the United States, residing at 6150 East Mercer Way, Mercer Island, Washington 98040 and CLAY SIEGALL, citizen of the United States, residing at 639 8th Avenue South, Edmonds, Washington 98020 ASSIGNORS, are the inventors of the invention in RECOMBINANT ANTI-CD30 ANTIBODIES AND USES THEREOF for which we have executed an application for a Patent of the United States

☐ which is executed on ☐ even date herewith or ☐ _____

☒ which is identified by Pennie & Edmonds LLP docket no. 9632-006-999
☒ which was filed on November 28, 2000, Application No. 09/724,406

and WHEREAS, Seattle Genetics, Inc., a corporation organized and existing under the laws of the State of Delaware, having its place of business at 22215 26th Avenue, S.E., Bothell, Washington 98021, ASSIGNEE, is desirous of obtaining our entire right, title and interest in, to and under the said invention and the said application:

NOW, THEREFORE, in consideration of the sum of One Dollar ($1.00) to us in hand paid, and other good and valuable consideration, the receipt of which is hereby acknowledged, we, the said ASSIGNORS, have sold, assigned, transferred and set over, and by these presents do hereby sell, assign, transfer and set over, unto the said ASSIGNEE, its successors, legal representatives and assigns, our entire right, title and interest in, to and under the said invention, and the said United States application and all divisions, renewals and continuations thereof, and all Patents of the United States which may be granted thereon and all reissues and extensions thereof; and all applications for industrial property protection, including, without limitation, all applications for patents, utility models, and designs which may hereafter be filed for said invention in any country or countries foreign to the United States, together with the right to file such applications and the right to claim for the same the priority rights derived from said United States application under the Patent Laws of the United States, the International Convention for the Protection of Industrial Property, or any other international agreement or the domestic laws of the country in which any such application is filed, as may be applicable; and all forms of industrial property protection, including, without limitation, patents, utility models, inventors' certificates and designs which may be granted for said invention in any country or countries foreign to the United States and all extensions, renewals and reissues thereof;

AND WE HEREBY authorize and request the Commissioner of Patents and Trademarks of the United States, and any Official of any country or countries foreign to the United States, whose duty it is to issue patents or other evidence or forms of industrial property protection on applications as aforesaid, to issue the same to the said ASSIGNEE, its successors, legal representatives and assigns, in accordance with the terms of this instrument.

AND WE HEREBY covenant and agree that we have full right to convey the entire interest herein assigned, and that we have not executed, and will not execute, any agreement in conflict herewith.

AND WE HEREBY further covenant and agree that we will communicate to the said ASSIGNEE, its successors, legal representatives and assigns, any facts known to us respecting said invention, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing, reissue and foreign applications, make all rightful oaths, and generally do everything possible to aid the said ASSIGNEE, its successors, legal representatives and assigns, to obtain and enforce proper protection for said invention in all countries.

IN TESTIMONY WHEREOF, We hereunto set our hands and seals the day and year set opposite our respective signatures.

Date January 10, 2001 _____ L.S.
JOSEPH A. FRANCISCO

State of Washington)
County of Snohomish) SS.:

On January 10, 2001, before me, Sandra K. Darling, Notary Public, personally appeared JOSEPH A. FRANCISCO, personally known to me on the basis of satisfactory evidence to be the person(s) whose name(s) is subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal

Sandra K. Darling

My commission expires: 1-29-02

Date 01-09-01, 2000 _____ L.S.
GRANT RISDON

State of MISSOURI }
County of ST LOUIS } SS.:

On January 9, 2001, before me, Margie Pinnell, Notary Public, personally appeared GRANT RISDON, personally known to me on the basis of satisfactory evidence to be the person(s) whose name(s) is subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal

Margie A. Pinnell

MARGIE A. PINNELL
Notary Public - Notary Seal
State of Missouri
Franklin County
My Commission Expires May 3, 2002

Date January 10, 2001 _____ L.S.
ALAN F. WAHL

State of Washington }
County of Snohomish } SS.:

On January 10, 2001, before me, Sandra K. Darling, Notary Public, personally appeared ALAN F. WAHL, personally known to me on the basis of satisfactory evidence to be the person(s) whose name(s) is subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal

Sandra K. Darling

My commission expires: 1-29-01

Date Jan. 10, 2001, 2000 _____ L.S.
CLAY SIEGALL

State of Washington }
County of Snohomish } SS.:

On January 10, 2001, before me, Sandra K. Darling, Notary Public, personally appeared CLAY SIEGALL, personally known to me on the basis of satisfactory evidence to be the person(s) whose name(s) is subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal

Sandra K. Darling

My commission expires: 1-29-02

2

PTO/SB/81A (12-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT - POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Patent Number	7,090,843
Issue Date	August 15, 2006
First Named Inventor	Joseph A. Francisco
Title	Recombinant Anti-CD30 Antibodies and Uses Thereof
Attorney Docket Number	0030-00101US

I hereby revoke all previous powers of attorney given in the above-identified patent.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith: 51946

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified patent to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☐ Inventor, having ownership of the patent.

OR

☒ Patent owner.

Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Inventor or Patent Owner

Signature		Date	09-30-2011
Name	Eric Dobmeier	Telephone	4255274000
Title and Company	Chief Operating Officer, Seattle Genetics, Inc.		

NOTE: Signatures of all the inventors or patent owners of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/96 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Seattle Genetics, Inc.

Application No./Patent No.: 7,090,843 Filed/Issue Date: August 15, 2006

Titled:
Recombinant Anti-CD30 Antibodies and Uses Thereof

Seattle Genetics, Inc. , a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;

2. ☐ an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or

3. ☐ the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 011508, Frame 0339, or for which a copy therefore is attached.

OR

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

E D/L — Signature
04-20-2011 — Date

Eric Dobmeier — Printed or Typed Name
Chief Operating Officer — Title

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL 125388/0
BL 125399/0

BLA ACCELERATED APPROVAL

August 19, 2011

SOME INFORMATION IN THIS DOCUMENT IS PROPRIETARY MATERIAL NOT OPEN TO PUBLIC. TO BE REVIEWED ONLY BY EXAMINER OR OTHER AUTHORIZED U.S. PATENT AND TRADEMARK OFFICE EMPLOYEE OR FDA EMPLOYEE

DO NOT SCAN

Seattle Genetics, Inc.
Attention: Elaine Waller
Senior Vice President, Regulatory Affairs
21823 30th Drive Southeast
Bothell, WA 98021

Dear Ms. Waller:

Please refer to your biologics license applications (BLAs) dated February 25, 2011, received February 28, 2011, submitted under section 351 of the Public Health Service Act for ADCETRIS (brentuximab vedotin).

We acknowledge receipt of your submissions dated March 14, 2011; March 17, 2011; March 22, 2011 and March 25, 2011, April 6, 2011, April 11, 2011, April 25, 2011, May 3, 2011, May 10, 2011, May 12, 2011, May 18, 2011, May 20, 2011, May 24, 2011, May 26, 2011, May 31, 2011, June 8, 2011, June 10, 2011, June 14, 2011, June 21, 2011, June 22, 2011, June 24, 2011, June 27, 2011, June 28, 2011, June 29, 2011, June 30, 2011, July 1, 2011, July 7, 2011, July 8, 2011, July 13, 2011, July 15, 2011, July 19, 2011, July 20, 2011, July 22, 2011, July 25, 2011, July 28, 2011, July 28, 2011, July 29, 2011, August 4, 2011, August 9, 2011, August 16, 2011, August 17, 2011 and August 17, 2011.

We are issuing Department of Health and Human Services U.S. License No. 1853 to Seattle Genetics, Inc., Bothell, WA under the provisions of section 351(a) of the Public Health Service Act controlling the manufacture and sale of biological products. The license authorizes you to introduce or deliver for introduction into interstate commerce, those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are authorized to manufacture the product brentuximab vedotin. Brentuximab vedotin is indicated for:

- The treatment of patients with Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates, and
- The treatment of patients with systemic anaplastic large cell lymphoma (sALCL) after failure of at least one prior multi-agent chemotherapy regimen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL 125388/0
BL 125399/0

BLA ACCELERATED APPROVAL

August 19, 2011

Seattle Genetics, Inc.
Attention: Elaine Waller
Senior Vice President, Regulatory Affairs
21823 30th Drive Southeast
Bothell, WA 98021

Dear Ms. Waller:

Please refer to your biologics license applications (BLAs) dated February 25, 2011, received February 28, 2011, submitted under section 351 of the Public Health Service Act for ADCETRIS (brentuximab vedotin).

We acknowledge receipt of your submissions dated March 14, 2011; March 17, 2011; March 22, 2011 and March 25, 2011, April 6, 2011, April 11, 2011, April 25, 2011, May 3, 2011, May 10, 2011, May 12, 2011, May 18, 2011, May 20, 2011, May 24, 2011, May 26, 2011, May 31, 2011, June 8, 2011, June 10, 2011, June 14, 2011, June 21, 2011, June 22, 2011, June 24, 2011, June 27, 2011, June 28, 2011, June 29, 2011, June 30, 2011, July 1, 2011, July 7, 2011, July 8, 2011, July 13, 2011, July 15, 2011, July 19, 2011, July 20, 2011, July 22, 2011, July 25, 2011, July 28, 2011, July 28, 2011, July 29, 2011, August 4, 2011, August 9, 2011, August 16, 2011, August 17, 2011 and August 17, 2011.

We are issuing Department of Health and Human Services U.S. License No. 1853 to Seattle Genetics, Inc., Bothell, WA under the provisions of section 351(a) of the Public Health Service Act controlling the manufacture and sale of biological products. The license authorizes you to introduce or deliver for introduction into interstate commerce, those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are authorized to manufacture the product brentuximab vedotin. Brentuximab vedotin is indicated for:

- The treatment of patients with Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates, and
- The treatment of patients with systemic anaplastic large cell lymphoma (sALCL) after failure of at least one prior multi-agent chemotherapy regimen.

Under this license, you are approved to manufacture cAC10 monoclonal antibody intermediate at Abbott Bioresearch Center, Inc. in Worcester, MA; SGD-1006 intermediate at SAFC Inc. in Madison, WI; and brentuximab vedotin drug substance at Piramal Healthcare (UK), Ltd. in Grangemouth Stirlingshire, UK. The drug product will be manufactured at Pierre Fabre Medicament Production Aquitaine Pharm. (PFMP) at Idron, France. You may label your product with the proprietary name ADCETRIS and will market it in a 50mg vial.

The dating period for brentuximab vedotin shall be 30 months from the date of manufacture when stored at 2-8°C (36-46°F) in the original carton. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for your cAC10 antibody intermediate shall be 36 months when stored at ≤60°C. We have approved the stability protocols in your license application for the purpose of extending the expiration dating period of your drug substance, drug product, and antibody intermediate under 21 CFR 601.12.

You currently are not required to submit samples of future lots of brentuximab vedotin to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of brentuximab vedotin, or in the manufacturing facilities.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

Because this biological product for this indication has an orphan drug designation, you are exempt from this requirement.

ACCELERATED APPROVAL REQUIREMENTS

As requested in your letter of February 25, 2011, marketing approval of this product is granted under the accelerated approval of biological products regulations, 21 CFR 601.40-46. These regulations permit the use of certain surrogate endpoints or an effect on a clinical endpoint other than survival or irreversible morbidity as bases for approvals of products intended for serious or life-threatening illnesses or conditions.

Approval under these regulations requires, among other things, that you conduct adequate and well-controlled studies/clinical trials to verify and describe clinical benefit attributable to this

product. You are required to conduct such trials with due diligence. If postmarketing trials fail to verify that clinical benefit is conferred by brentuximab vedotin, or are not conducted with due diligence, we may, following a hearing in accordance with 21 CFR 601.43(b), withdraw or modify approval.

Granting of these approvals are contingent upon completion of clinical trials to verify the clinical benefit of brentuximab vedotin, as outlined in your letter of July 22, 2011. These postmarketing trials are subject to the reporting requirements of 21 CFR 601.70:

1. **CONFIRMATORY TRIAL**- A randomized phase 3, double-blind, placebo-controlled trial of SGN-35 (brentuximab vedotin) in combination with CH-P versus CHOP as frontline therapy in patients with CD30-positive mature T- and NK-cell lymphomas including systemic ALCL (sALCL). Enrollment of approximately 300 patients is expected with a primary endpoint of progression free survival as determined by an independent blinded review facility. Overall survival is a key secondary endpoint.

   Final Protocol Submission Date: 3/2013
   Trial Completion Date: 3/2019
   Final Report Submission Date: 9/2019

2. **CONFIRMATORY TRIAL** - A randomized phase 3 trial of SGN-35 (brentuximab vedotin) in combination with AVD versus ABVD as frontline therapy in patients with advanced Hodgkin Lymphoma. Enrollment of at least 880 patients is expected with a primary endpoint of progression free survival determined by an independent blinded review facility. Overall survival is a key secondary endpoint.

   Final Protocol Submission Date: 09/2012
   Trial Completion Date: 12/2018
   Final Report Submission Date: 06/2019

Successful completion of either PMR 1 or PMR 2 could be considered to convert the accelerated approval to regular approval for both the Hodgkin lymphoma and sALCL indications.

We expect you to complete design, initiation, accrual, completion, and reporting of these trials within the framework described in your letter of August 10, 2011.

For administrative purposes, all submissions related to this/these postmarketing trials should be clearly designated "Subpart E Postmarketing Trial Requirements".

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess a known serious risk of neuropathy with ADCETRIS therapy.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA will not be sufficient to assess this serious risk.

Finally, we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to assess a known serious risk of neuropathy with ADCETRIS therapy.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

3. **FOR BLAs 125388 & 125399- Reversibility/Resolution of drug-induced peripheral neuropathy**. Characterize the severity, duration and reversibility of treatment emergent neuropathy in a prospective trial.

   The ongoing placebo-controlled AETHERA trial safety results may be utilized to address this PMR.

   Phase 3 Trial Completion Date: 12/2013
   Phase 3 Trial Final Report Submission Date: 6/2014

Submit the protocol to your IND 071635, with a cross-reference letter to this BLA. Submit all final report(s) to your BLA. Prominently identify the submission with the following wording in bold capital letters at the top of the first page of the submission, as appropriate: **"Required Postmarketing Protocol Under 505(o)", "Required Postmarketing Final Report Under 505(o)", "Required Postmarketing Correspondence Under 505(o)".**

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies

or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS NOT SUBJECT TO REPORTING REQUIREMENTS OF 21 CFR 601.70

In addition, we acknowledge your written commitments as described in your letter of August 10, 2011, as outlined below.

4. Perform additional experimental work to understand the impact of soluble CD30 in serum samples on the determination of anti-drug antibodies.

   Final Report Submission Date: 09/2012

5. Provide summary data for validating all in-process product intermediate maximum hold times for the cAC10 manufacturing process at scale in a CBE0.

   Final Report Submission Date: 12/2012

6. Perform the bacteriostasis/fungistasis testing for the bioburden test of the bulk drug substance using three batches of BDS samples stored under routine sample storage conditions at 2-8°C. Summary data should be submitted in the next Annual Report.

   Final Report Submission Date: 12/2012

7. Commit to reassess brentuximab vedotin drug substance and drug product specifications based on the combination of Intermediate lots used to manufacture SGN-35 BDS and DP when the total number of BDS and DP lots include ≥25 lots cAC10 and ≥10 lots of SGD-1006 as input intermediates and, as part of your annual Product Quality Review for brentuximab vedotin.

   Final Report Submission Date: 03/2016

8. Harmonize all CMC information contained in your application with that contained in DMF 22144.

   Final Report Submission Date: 11/2011

9. Reevaluate the Limit of Detection (LOD) of methylene blue using standard curve with different concentrations of dye that include concentrations below the LOD. Results of the LOD determination will be appended to the method validation report.

   Final Report Submission Date: 12/2011

10. The CDRH guidance referenced for biological indicator (BI) incubation time has been superseded by the CDRH Guidance on BI Premarket Notification 510(k) Submissions.

The guidance refers to BIs used to monitor sterilization processes in health care facilities. BIs intended for use in a manufacturing setting are excluded. The EZ Test BIs used for steam sterilization validation studies should be incubated for a full 7 days to confirm that all BIs are negative. This change should be made to the autoclave and SIP validation protocols at PFMP and reported in the next annual report.

Final Report Submission Date: 12/2012

Submit clinical protocols to your IND, with a cross-reference letter to these biologics license applications (BLAs), STN BL [125388/0] and [125399/0]. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to your BLAs, STN BL [125388/0] and STN BL [125399/0]. Use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **POSTMARKETING COMMITMENT PROTOCOL**
- **POSTMARKETING COMMITMENT - FINAL STUDY REPORT**
- **POSTMARKETING COMMITMENT CORRESPONDENCE**
- **ANNUAL STATUS REPORT OF POSTMARKETING COMMITMENTS**

For each postmarketing commitment subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies/clinical trials for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

ADVERSE EVENT REPORTING

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Hematology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
5901-B Ammendale Road
Beltsville, MD 20705-1266

Biological product deviations sent by courier or overnight mail should be addressed to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
10903 New Hampshire Avenue, Bldg. 51, Room 4206
Silver Spring, MD 20903

CONTENT OF LABELING

Within 14 days of the date of this letter, submit content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm that is identical in content to the enclosed labeling text. The content of labeling should be submitted by updating your application by referencing the SPL file submitted to the drug establishment registration and drug listing system. To do this, place a link in your application submission that directs FDA to your SPL file. For administrative purposes, please designate this submission **"Product Correspondence – Final SPL for approved STN BLAs 125388/0 and 125399/0."**

In addition, within 14 days of the date of this letter, amend any pending supplement for this BLA with content of labeling in SPL format to include the changes approved in this supplement. For additional information on submitting labeling to drug establishment registration and drug listing and to applications, see the FDA guidances at

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072339.pdf and
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf.

We are waiving the requirements of 21 CFR 201.57(d)(8) regarding the length of Highlights of prescribing information. This waiver applies to all future supplements containing revised labeling unless we notify you otherwise.

CARTON AND CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed draft labels as soon as they are available but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005).* Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission **"Product Correspondence – Final Printed Carton and Container Labels for approved STN BL 125388/0 and 125399/0."** Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with labeling that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

PROMOTIONAL MATERIALS

Immediately submit all promotional materials (both promotional labeling and advertisements) to be used within the first 120 days after approval. Send one copy to this division, the Division of HEMATOLOGY PRODUCTS, and two copies of the promotional materials and the package insert directly to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

In addition, as required by 21 CFR 601.45, submit all subsequent promotional materials at least 30 days before the intended time of initial distribution of labeling or initial publication of the advertisement. Send two copies of the promotional materials and the package insert to the address above.

POST-ACTION FEEDBACK MEETING

New molecular entities and important new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during the drug development and marketing application review process. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, contact the Division of Hematology Products.

If you have any questions, contact the Regulatory Project Manager, Lara Akinsanya, at (301) 796-9634.

Sincerely,

Richard Pazdur MD

/Richard Pazdur, M.D./
Richard Pazdur, M.D.
Office Director
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure
Content of Labeling
Carton and Container Labeling

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use ADCETRIS safely and effectively. See full prescribing information for ADCETRIS.

ADCETRIS™ (brentuximab vedotin) for Injection

For Intravenous infusion

Initial U.S. approval: 2011

INDICATIONS AND USAGE

ADCETRIS is a CD30-directed antibody-drug conjugate indicated for:

- The treatment of patients with Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates (1.1).
- The treatment of patients with systemic anaplastic large cell lymphoma after failure of at least one prior multi-agent chemotherapy regimen (1.2).

These indications are based on response rate. There are no data available demonstrating improvement in patient reported outcomes or survival with ADCETRIS.

DOSAGE AND ADMINISTRATION

- The recommended dose is 1.8 mg/kg administered only as an intravenous infusion over 30 minutes every 3 weeks (2).
- Continue treatment until a maximum of 16 cycles, disease progression or unacceptable toxicity.

DOSAGE FORMS AND STRENGTHS

50 mg single-use vial (3).

CONTRAINDICATIONS

None (4).

WARNINGS AND PRECAUTIONS

- Peripheral neuropathy: Treating physicians should monitor patients for neuropathy and institute dose modifications accordingly (5.1).
- Infusion reactions: If an infusion reaction occurs, the infusion should be interrupted and appropriate medical management instituted. If anaphylaxis occurs, the infusion should be discontinued immediately and appropriate medical management instituted (5.2).
- Neutropenia: Monitor complete blood counts prior to each dose of ADCETRIS. If Grade 3 or 4 neutropenia develops, manage by dose delays, reductions or discontinuation (5.3).
- Tumor Lysis Syndrome: Patients with rapidly proliferating tumor and high tumor burden are at risk of tumor lysis syndrome and these patients should be monitored closely and appropriate measures taken (5.4).
- Stevens-Johnson syndrome: If Stevens-Johnson syndrome occurs, discontinue ADCETRIS and administer appropriate medical therapy (5.5).
- Progressive Multifocal Leukoencephalopathy (PML): A fatal case of PML has been reported in a patient who received 4 chemotherapy regimens prior to receiving ADCETRIS (5.6).
- Use in pregnancy: Fetal harm can occur. Pregnant women should be advised of the potential hazard to the fetus (5.7).

ADVERSE REACTIONS

The most common adverse reactions (≥20%) are neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia, cough, and vomiting (6.1).

To report SUSPECTED ADVERSE REACTIONS, contact Seattle Genetics, Inc. at 1-855-473-2436 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Patients who are receiving strong CYP3A4 inhibitors concomitantly with ADCETRIS should be closely monitored for adverse reactions (7.1).

USE IN SPECIFIC POPULATIONS

None (8).

See 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS

1 INDICATIONS AND USAGE
- 1.1 Hodgkin Lymphoma
- 1.2 Systemic Anaplastic Large Cell Lymphoma

2 DOSAGE AND ADMINISTRATION
- 2.1 General Dosing Information
- 2.2 Dose Modification
- 2.3 Instructions for Preparation and Administration

3 DOSAGE FORMS AND STRENGTHS

4 CONTRAINDICATIONS

5 WARNINGS AND PRECAUTIONS
- 5.1 Peripheral Neuropathy
- 5.2 Infusion Reactions
- 5.3 Neutropenia
- 5.4 Tumor Lysis Syndrome
- 5.5 Stevens-Johnson Syndrome
- 5.6 Progressive Multifocal Leukoencephalopathy
- 5.7 Use in Pregnancy

6 ADVERSE REACTIONS
- 6.1 Clinical Trial Experience
- 6.2 Immunogenicity

7 DRUG INTERACTIONS
- 7.1 Effect of Other Drugs on ADCETRIS
- 7.2 Effect of ADCETRIS on Other Drugs

8 USE IN SPECIFIC POPULATIONS
- 8.1 Pregnancy
- 8.3 Nursing Mothers
- 8.4 Pediatric Use
- 8.5 Geriatric Use
- 8.6 Renal Impairment
- 8.7 Hepatic Impairment

10 OVERDOSAGE

11 DESCRIPTION

12 CLINICAL PHARMACOLOGY
- 12.1 Mechanism of Action
- 12.2 Pharmacodynamics
- 12.3 Pharmacokinetics

13 NONCLINICAL TOXICOLOGY
- 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

14 CLINICAL STUDIES
- 14.1 Hodgkin Lymphoma
- 14.2 Systemic Anaplastic Large Cell Lymphoma

15 REFERENCES

16 HOW SUPPLIED/STORAGE AND HANDLING
- 16.1 How Supplied
- 16.2 Storage
- 16.3 Special Handling

17 PATIENT COUNSELING INFORMATION

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

These indications are based on response rate. There are no data available demonstrating improvement in patient reported outcomes or survival with ADCETRIS.

1.1 Hodgkin Lymphoma

ADCETRIS (brentuximab vedotin) is indicated for treatment of patients with Hodgkin lymphoma (HL) after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates.

1.2 Systemic Anaplastic Large Cell Lymphoma

ADCETRIS is indicated for treatment of patients with systemic anaplastic large cell lymphoma (sALCL) after failure of at least one prior multi-agent chemotherapy regimen.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

The recommended dose is 1.8 mg/kg administered only as an intravenous infusion over 30 minutes every 3 weeks.

Do not administer as an intravenous push or bolus.

Continue treatment until a maximum of 16 cycles, disease progression or unacceptable toxicity.

2.2 Dose Modification

Peripheral Neuropathy: Peripheral neuropathy should be managed using a combination of dose delay and reduction to 1.2 mg/kg. For new or worsening Grade 2 or 3 neuropathy, dosing should be held until neuropathy improves to Grade 1 or baseline and then restarted at 1.2 mg/kg. For Grade 4 peripheral neuropathy, ADCETRIS should be discontinued.

Neutropenia: Neutropenia should be managed by dose delays and reductions. The dose of ADCETRIS should be held for Grade 3 or 4 neutropenia until resolution to baseline or Grade 2 or lower. Growth factor support should be considered for subsequent cycles in patients who experience Grade 3 or 4 neutropenia. In patients with recurrent Grade 4 neutropenia despite the use of growth factors, discontinuation or dose reduction of ADCETRIS to 1.2 mg/kg may be considered.

2.3 Instructions for Preparation and Administration

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published [*see References (15)*].

Use appropriate aseptic technique for reconstitution and preparation of dosing solutions.

Reconstitution

Calculate the dose (mg) and number of vials of ADCETRIS required. The dose for patients with a weight of >100 kg should be calculated for 100 kg. Reconstitute each 50 mg vial of ADCETRIS with 10.5 mL of Sterile Water for Injection, USP, to yield a single-use solution containing 5 mg/mL brentuximab vedotin. Direct the stream toward wall of vial and not directly at the cake or powder. Gently swirl the vial to aid dissolution. **DO NOT SHAKE**. Inspect the reconstituted solution for particulates and discoloration. The reconstituted solution should be clear to slightly opalescent, colorless, and free of visible particulates. Following reconstitution, dilute immediately into an infusion bag, or store the solution at 2-8°C (36-46°F) and use within 24 hours of reconstitution. **DO NOT FREEZE.** Discard any unused portion left in the vial.

Dilution

Calculate the required volume of 5 mg/mL reconstituted ADCETRIS solution needed and withdraw this amount from the vials. The dose for patients with a weight of >100 kg should be calculated for 100 kg. Immediately add the reconstituted solution to an infusion bag containing a minimum volume of 100 mL to achieve a final concentration of 0.4 mg/mL to 1.8 mg/mL brentuximab vedotin. ADCETRIS can be diluted into 0.9% Sodium Chloride Injection, 5% Dextrose Injection or Lactated Ringer's Injection. Gently invert the bag to mix the solution. ADCETRIS contains no bacteriostatic preservatives. Following dilution, infuse the ADCETRIS solution immediately, or store the solution at 2-8°C (36-46°F) and use within 24 hours of reconstitution. **DO NOT FREEZE.**

Do not mix ADCETRIS with, or administer as an infusion with, other medicinal products.

3 DOSAGE FORMS AND STRENGTHS

ADCETRIS (brentuximab vedotin) for Injection single-use vial containing 50 mg of brentuximab vedotin as a sterile, white to off-white lyophilized, preservative-free cake or powder.

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Peripheral Neuropathy

ADCETRIS treatment causes a peripheral neuropathy that is predominantly sensory. Cases of peripheral motor neuropathy have also been reported. ADCETRIS-induced peripheral neuropathy is cumulative. In the HL and sALCL clinical trials, 54% of patients experienced any grade of neuropathy. Of these patients, 49% had complete resolution, 31% had partial improvement, and 20% had no improvement. Of the patients who reported neuropathy, 51% had residual neuropathy at the time of their last evaluation. Monitor patients for symptoms of neuropathy, such as hypoesthesia, hyperesthesia, paresthesia, discomfort, a burning sensation, neuropathic pain or weakness. Patients experiencing new or worsening peripheral neuropathy may require a delay, change in dose, or discontinuation of ADCETRIS [see *Dose Modification (2.2)*].

5.2 Infusion Reactions

Infusion-related reactions, including anaphylaxis, have occurred with ADCETRIS. Monitor patients during infusion. If anaphylaxis occurs, immediately and permanently discontinue administration of ADCETRIS and administer appropriate medical therapy. If an infusion-related reaction occurs, the infusion should be interrupted and appropriate medical management instituted. Patients who have experienced a prior infusion-related reaction should be premedicated for subsequent infusions. Premedication may include acetaminophen, an antihistamine and a corticosteroid.

5.3 Neutropenia

Complete blood counts should be monitored prior to each dose of ADCETRIS and more frequent monitoring should be considered for patients with Grade 3 or 4 neutropenia. Prolonged (≥1 week) severe neutropenia can occur with ADCETRIS. If Grade 3 or 4 neutropenia develops, manage by dose delays, reductions, or discontinuations [*see Dose Modification (2.2)*].

5.4 Tumor Lysis Syndrome

Tumor lysis syndrome may occur. Patients with rapidly proliferating tumor and high tumor burden may be at increased risk of tumor lysis syndrome. Monitor closely and take appropriate measures.

5.5 Stevens-Johnson Syndrome

Stevens-Johnson syndrome has been reported with ADCETRIS. If Stevens-Johnson syndrome occurs, discontinue ADCETRIS and administer appropriate medical therapy.

5.6 Progressive Multifocal Leukoencephalopathy

A fatal case of progressive multifocal leukoencephalopathy (PML) has been reported in a patient who received 4 chemotherapy regimens prior to receiving ADCETRIS.

5.7 Use in Pregnancy

There are no adequate and well-controlled studies of ADCETRIS in pregnant women. However, based on its mechanism of action and findings in animals, ADCETRIS can cause fetal harm when administered to a pregnant woman. Brentuximab vedotin caused embryo-fetal toxicities, including significantly decreased embryo viability and fetal malformations, in animals at maternal exposures that were similar to human exposures at the recommended doses for patients with HL and sALCL. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving the drug, the patient should be apprised of the potential hazard to the fetus *[see Use in Specific Populations (8.1)]*.

6 ADVERSE REACTIONS

6.1 Clinical Trial Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

ADCETRIS was studied as monotherapy in 160 patients in two phase 2 trials. Across both trials, the most common adverse reactions (≥20%), regardless of causality, were neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia, cough and vomiting. The most common adverse reactions occurring in at least 10% of patients in either trial, regardless of causality, using the NCI Common Toxicity Criteria Version 3.0, are shown in Table 1.

Experience in Hodgkin Lymphoma

ADCETRIS was studied in 102 patients with HL in a single arm clinical trial in which the recommended starting dose and schedule was 1.8 mg/kg intravenously every 3 weeks. Median duration of treatment was 27 weeks (range, 3 to 56 weeks) [*see Clinical Studies (14)*].

The most common adverse reactions (≥20%), regardless of causality, were neutropenia, peripheral sensory neuropathy, fatigue, upper respiratory tract infection, nausea, diarrhea, anemia, pyrexia, thrombocytopenia, rash, abdominal pain, cough, and vomiting.

Experience in Systemic Anaplastic Large Cell Lymphoma

ADCETRIS was studied in 58 patients with sALCL in a single arm clinical trial in which the recommended starting dose and schedule was 1.8 mg/kg intravenously every 3 weeks. Median duration of treatment was 24 weeks (range, 3 to 56 weeks) [*see Clinical Studies (14)*].

The most common adverse reactions (≥20%), regardless of causality, were neutropenia, anemia, peripheral sensory neuropathy, fatigue, nausea, pyrexia, rash, diarrhea, and pain.

Combined Experience

Table 1: Most Commonly Reported (≥10%) Adverse Reactions

	HL Total N = 102 % of patients			sALCL Total N = 58 % of patients		
Adverse Reaction	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
Blood and lymphatic system disorders						
Neutropenia*	54	15	6	55	12	9
Anemia*	33	8	2	52	2	-
Thrombocytopenia*	28	7	2	16	5	5
Lymphadenopathy	11	-	-	10	-	-
Nervous system disorders						
Peripheral sensory neuropathy	52	8	-	53	10	-
Peripheral motor neuropathy	16	4	-	7	3	-
Headache	19	-	-	16	2	-
Dizziness	11	-	-	16	-	-

Adverse Reaction	HL Total N = 102 % of patients			sALCL Total N = 58 % of patients		
	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
General disorders and administration site conditions						
Fatigue	49	3	-	41	2	2
Pyrexia	29	2	-	38	2	-
Chills	13	-	-	12	-	-
Pain	7	-	-	28	-	5
Edema peripheral	4	-	-	16	-	-
Infections and infestations						
Upper respiratory tract infection	47	-	-	12	-	-
Gastrointestinal disorders						
Nausea	42	-	-	38	2	-
Diarrhea	36	1	-	29	3	-
Abdominal pain	25	2	1	9	2	-
Vomiting	22	-	-	17	3	-
Constipation	16	-	-	19	2	-
Skin and subcutaneous tissue disorders						
Rash	27	-	-	31	-	-
Pruritus	17	-	-	19	-	-
Alopecia	13	-	-	14	-	-
Night sweats	12	-	-	9	-	-
Dry skin	4	-	-	10	-	-
Respiratory, thoracic and mediastinal disorders						
Cough	25	-	-	17	-	-
Dyspnea	13	1	-	19	2	-
Oropharyngeal pain	11	-	-	9	-	-
Musculoskeletal and connective tissue disorders						
Arthralgia	19	-	-	9	-	-
Myalgia	17	-	-	16	2	-
Back pain	14	-	-	10	2	-
Pain in extremity	10	-	-	10	2	2
Muscle spasms	9	-	-	10	2	-
Psychiatric disorders						
Insomnia	14	-	-	16	-	-
Anxiety	11	2	-	7	-	-

antibodies (positive test at more than 2 timepoints) and 30% developed transiently positive antibodies (positive in 1 or 2 post-baseline timepoints). The anti-brentuximab antibodies were directed against the antibody component of brentuximab vedotin in all patients with transiently or persistently positive antibodies. Two of the patients (1%) with persistently positive antibodies experienced adverse reactions consistent with infusion reactions that led to discontinuation of treatment. Overall, a higher incidence of infusion related reactions was observed in patients who developed persistently positive antibodies.

A total of 58 patient samples that were either transiently or persistently positive for anti-brentuximab vedotin antibodies were tested for the presence of neutralizing antibodies. Sixty-two percent of these patients had at least one sample that was positive for the presence of neutralizing antibodies. The effect of anti-brentuximab vedotin antibodies on safety and efficacy is not known.

Immunogenicity assay results are highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to ADCETRIS with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

In vitro data indicate that monomethyl auristatin E (MMAE) is a substrate and an inhibitor of CYP3A4/5.

7.1 Effect of Other Drugs on ADCETRIS

CYP3A4 Inhibitors/Inducers: MMAE is primarily metabolized by CYP3A [*see Clinical Pharmacology (12.3)*]. Co-administration of ADCETRIS with ketoconazole, a potent CYP3A4 inhibitor, increased exposure to MMAE by approximately 34%. Patients who are receiving strong CYP3A4 inhibitors concomitantly with ADCETRIS should be closely monitored for adverse reactions. Co-administration of ADCETRIS with rifampin, a potent CYP3A4 inducer, reduced exposure to MMAE by approximately 46%.

7.2 Effect of ADCETRIS on Other Drugs

Co-administration of ADCETRIS did not affect exposure to midazolam, a CYP3A4 substrate. MMAE does not inhibit other CYP enzymes at relevant clinical concentrations [*see Clinical Pharmacology (12.3)*]. ADCETRIS is not expected to alter the exposure to drugs that are metabolized by CYP3A4 enzymes.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D *[see Warnings and Precautions (5.7)]*.

There are no adequate and well-controlled studies with ADCETRIS in pregnant women. However, based on its mechanism of action and findings in animals, ADCETRIS can cause fetal harm when administered to a pregnant woman. Brentuximab vedotin caused embryo-fetal

toxicities in animals at maternal exposures that were similar to human exposures at the recommended doses for patients with HL and sALCL. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus.

In an embryo-fetal developmental study, pregnant rats received 2 intravenous doses of 0.3, 1, 3, or 10 mg/kg brentuximab vedotin during the period of organogenesis (once each on Pregnancy Days 6 and 13). Drug-induced embryo-fetal toxicities were seen mainly in animals treated with 3 and 10 mg/kg of the drug and included increased early resorption (≥99%), post-implantation loss (≥99%), decreased numbers of live fetuses, and external malformations (i.e., umbilical hernias and malrotated hindlimbs). Systemic exposure in animals at the brentuximab vedotin dose of 3 mg/kg is approximately the same exposure in patients with HL or sALCL who received the recommended dose of 1.8 mg/kg every three weeks.

8.3 Nursing Mothers

It is not known whether brentuximab vedotin is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from ADCETRIS a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

The safety and effectiveness of ADCETRIS have not been established in the pediatric population. Clinical trials of ADCETRIS included only 9 pediatric patients and this number is not sufficient to determine whether they respond differently than adult patients.

8.5 Geriatric Use

Clinical trials of ADCETRIS did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. Safety and efficacy have not been established.

8.6 Renal Impairment

The kidney is a route of excretion for MMAE. The influence of renal impairment on the pharmacokinetics of MMAE has not been determined.

8.7 Hepatic Impairment

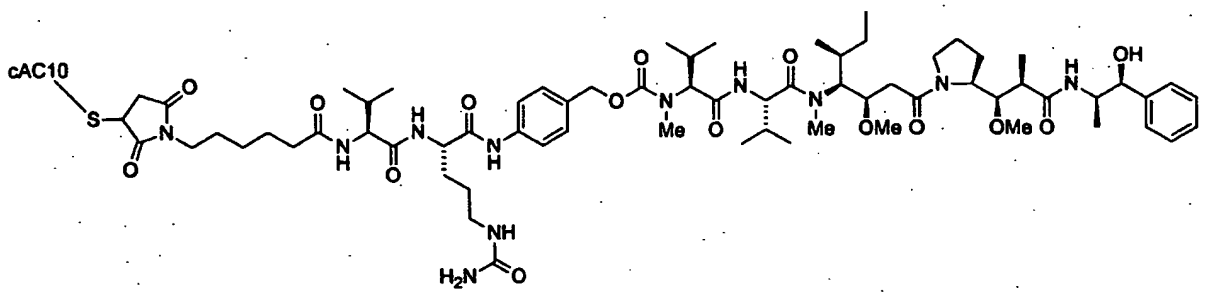
The liver is a route of clearance for MMAE. The influence of hepatic impairment on the pharmacokinetics of MMAE has not been determined.

10 OVERDOSAGE

There is no known antidote for overdosage of ADCETRIS. In case of overdosage, the patient should be closely monitored for adverse reactions, particularly neutropenia, and supportive treatment should be administered.

11 DESCRIPTION

ADCETRIS (brentuximab vedotin) is a CD30-directed antibody-drug conjugate (ADC) consisting of three components: 1) the chimeric IgG1 antibody cAC10, specific for human CD30, 2) the microtubule disrupting agent MMAE, and 3) a protease-cleavable linker that covalently attaches MMAE to cAC10.



Brentuximab vedotin has an approximate molecular weight of 153 kDa. Approximately 4 molecules of MMAE are attached to each antibody molecule. Brentuximab vedotin is produced by chemical conjugation of the antibody and small molecule components. The antibody is produced by mammalian (Chinese hamster ovary) cells, and the small molecule components are produced by chemical synthesis.

ADCETRIS (brentuximab vedotin) for Injection is supplied as a sterile, white to off-white, preservative-free lyophilized cake or powder in single-use vials. Following reconstitution with 10.5 mL Sterile Water for Injection, USP, a solution containing 5 mg/mL brentuximab vedotin is produced. The reconstituted product contains 70 mg/mL trehalose dihydrate, 5.6 mg/mL sodium citrate dihydrate, 0.21 mg/mL citric acid monohydrate, and 0.20 mg/mL polysorbate 80 and water for injection. The pH is approximately 6.6.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Brentuximab vedotin is an ADC. The antibody is a chimeric IgG1 directed against CD30. The small molecule, MMAE, is a microtubule disrupting agent. MMAE is covalently attached to the antibody via a linker. Nonclinical data suggest that the anticancer activity of ADCETRIS is due to the binding of the ADC to CD30-expressing cells, followed by internalization of the ADC-CD30 complex, and the release of MMAE via proteolytic cleavage. Binding of MMAE to tubulin disrupts the microtubule network within the cell, subsequently inducing cell cycle arrest and apoptotic death of the cells.

12.2 Pharmacodynamics

QT/QTc Prolongation Potential

The effect of brentuximab vedotin (1.8 mg/kg) on the QTc interval was evaluated in an open-label, single-arm study in 46 evaluable patients with CD30-expressing hematologic malignancies. Administration of brentuximab vedotin did not prolong the mean QTc interval

>10 ms from baseline. Small increases in the mean QTc interval (<10 ms) cannot be excluded because this study did not include a placebo arm and a positive control arm.

12.3 Pharmacokinetics

The pharmacokinetics of brentuximab vedotin were evaluated in phase 1 trials and in a population pharmacokinetic analysis of data from 314 patients. The pharmacokinetics of three analytes were determined: the ADC, MMAE, and total antibody. Total antibody had the greatest exposure and had a similar PK profile as the ADC. Hence, data on the PK of the ADC and MMAE have been summarized.

Absorption

Maximum concentrations of ADC were typically observed close to the end of infusion. A multiexponential decline in ADC serum concentrations was observed with a terminal half-life of approximately 4 to 6 days. Exposures were approximately dose proportional from 1.2 to 2.7 mg/kg. Steady-state of the ADC was achieved within 21 days with every 3-week dosing of ADCETRIS, consistent with the terminal half-life estimate. Minimal to no accumulation of ADC was observed with multiple doses at the every 3-week schedule.

The time to maximum concentration for MMAE ranged from approximately 1 to 3 days. Similar to the ADC, steady-state of MMAE was achieved within 21 days with every 3 week dosing of ADCETRIS. MMAE exposures decreased with continued administration of ADCETRIS with approximately 50% to 80% of the exposure of the first dose being observed at subsequent doses.

Distribution

In vitro, the binding of MMAE to human plasma proteins ranged from 68-82%. MMAE is not likely to displace or to be displaced by highly protein-bound drugs. In vitro, MMAE was a substrate of P-gp and was not a potent inhibitor of P-gp.

In humans, the mean steady state volume of distribution was approximately 6-10 L for ADC.

Metabolism

In vivo data in animals and humans suggest that only a small fraction of MMAE released from brentuximab vedotin is metabolized. In vitro data indicate that the MMAE metabolism that occurs is primarily via oxidation by CYP3A4/5. In vitro studies using human liver microsomes indicate that MMAE inhibits CYP3A4/5 but not other CYP isoforms. MMAE did not induce any major CYP450 enzymes in primary cultures of human hepatocytes.

Elimination

MMAE appeared to follow metabolite kinetics, with the elimination of MMAE appearing to be limited by its rate of release from ADC. An excretion study was undertaken in patients who received a dose of 1.8 mg/kg of ADCETRIS. Approximately 24% of the total MMAE administered as part of the ADC during an ADCETRIS infusion was recovered in both urine and feces over a 1-week period. Of the recovered MMAE, approximately 72% was recovered in the feces and the majority of the excreted MMAE was unchanged.

Effects of Gender, Age and Race

Based on the population pharmacokinetic analysis, gender, age and race do not have a meaningful effect on the pharmacokinetics of brentuximab vedotin.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies with brentuximab vedotin or the small molecule (MMAE) have not been conducted.

MMAE was genotoxic in the rat bone marrow micronucleus study through an aneugenic mechanism. This effect is consistent with the pharmacological effect of MMAE as a microtubule disrupting agent. MMAE was not mutagenic in the bacterial reverse mutation assay (Ames test) or the L5178Y mouse lymphoma forward mutation assay.

Fertility studies with brentuximab vedotin or MMAE have not been conducted. However, results of repeat-dose toxicity studies in rats indicate the potential for brentuximab vedotin to impair male reproductive function and fertility. In a 4-week repeat-dose toxicity study in rats with weekly dosing at 0.5, 5 or 10 mg/kg brentuximab vedotin, seminiferous tubule degeneration, Sertoli cell vacuolation, reduced spermatogenesis and aspermia were observed. Effects in animals were seen mainly at 5 and 10 mg/kg of brentuximab vedotin. These doses are approximately 3 and 6-fold the human recommended dose of 1.8 mg/kg, respectively, based on body weight.

14 CLINICAL STUDIES

14.1 Hodgkin Lymphoma

The efficacy of ADCETRIS in patients with HL who relapsed after autologous stem cell transplant was evaluated in one open-label, single-arm, multicenter trial. One hundred two patients were treated with 1.8 mg/kg of ADCETRIS intravenously over 30 minutes every 3 weeks. An independent review facility performed efficacy evaluations which included overall response rate (ORR = complete remission [CR] + partial remission [PR]) and duration of response as defined by clinical and radiographic measures including computed tomography (CT) and positron-emission tomography (PET) as defined in the 2007 Revised Response Criteria for Malignant Lymphoma (modified).

The 102 patients ranged in age from 15-77 years (median, 31 years) and most were female (53%) and white (87%). Patients had received a median of 5 prior therapies including autologous stem cell transplant.

The efficacy results are summarized in Table 2. Duration of response is calculated from date of first response to date of progression or data cutoff date.

Table 2: Efficacy Results in Patients with Hodgkin Lymphoma

	N=102		
	Percent (95%CI)	Duration of Response, in months	
		Median (95% CI)	Range
CR	32 (23, 42)	20.5 (12.0, NE*)	1.4 to 21.9+
PR	40 (32, 49)	3.5 (2.2, 4.1)	1.3 to 18.7
ORR	73 (65, 83)	6.7 (4.0, 14.8)	1.3 to 21.9+

*Not estimable

+ Follow up was ongoing at the time of data submission.

14.2 Systemic Anaplastic Large Cell Lymphoma

The efficacy of ADCETRIS in patients with relapsed sALCL was evaluated in one phase 2 open-label, single-arm, multicenter trial. This trial included patients who had sALCL that was relapsed after prior therapy. Fifty-eight patients were treated with 1.8 mg/kg of ADCETRIS administered intravenously over 30 minutes every 3 weeks. An independent review facility performed efficacy evaluations which included overall response rate (ORR = complete remission [CR] + partial remission [PR]) and duration of response as defined by clinical and radiographic measures including computed tomography (CT) and positron-emission tomography (PET) as defined in the 2007 Revised Response Criteria for Malignant Lymphoma (modified).

The 58 patients ranged in age from 14-76 years (median, 52 years) and most were male (57%) and white (83%). Patients had received a median of 2 prior therapies; 26% of patients had received prior autologous stem cell transplant. Fifty percent (50%) of patients were relapsed and 50% of patients were refractory to their most recent prior therapy. Seventy-two percent (72%) were anaplastic lymphoma kinase (ALK)-negative.

The efficacy results are summarized in Table 3. Duration of response is calculated from date of first response to date of progression or data cutoff date.

Table 3: Efficacy Results in Patients with Systemic Anaplastic Large Cell Lymphoma

	N=58		
	Percent (95%CI)	Duration of Response, in months	
		Median (95% CI)	Range
CR	57 (44, 70)	13.2 (10.8, NE*)	0.7 to 15.9+
PR	29 (18, 41)	2.1 (1.3, 5.7)	0.1 to 15.8+
ORR	86 (77, 95)	12.6 (5.7, NE*)	0.1 to 15.9+

*Not estimable

+ Follow up was ongoing at the time of data submission.

15 REFERENCES

1. NIOSH Alert: Preventing occupational exposure to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165
2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. Am J Health-Syst Pharm. (2006) 63:1172-1193
4. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

ADCETRIS (brentuximab vedotin) for Injection is supplied as a sterile, white to off-white preservative-free lyophilized cake or powder in individually-boxed single-use vials:

- **NDC (51144-050-01), 50 mg brentuximab vedotin.**

16.2 Storage

Store vial at 2-8°C (36-46°F) in the original carton to protect from light.

16.3 Special Handling

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published[1-4].

17 PATIENT COUNSELING INFORMATION

• Peripheral neuropathy

Advise patients that ADCETRIS can cause a peripheral neuropathy. They should be advised to report to their health care provider any numbness or tingling of the hands or feet or any muscle weakness [*see Warnings and Precautions (5.1)*].

• Fever/Neutropenia

Advise patients to contact their health care provider if a fever of 100.5°F or greater or other evidence of potential infection such as chills, cough, or pain on urination develops [*see Warnings and Precautions (5.3)*].

• **Infusion reactions**

Advise patients to contact their health care provider if they experience signs and symptoms of infusion reactions including fever, chills, rash, or breathing problems within 24 hours of infusion [*see Warnings and Precautions (5.2)*].

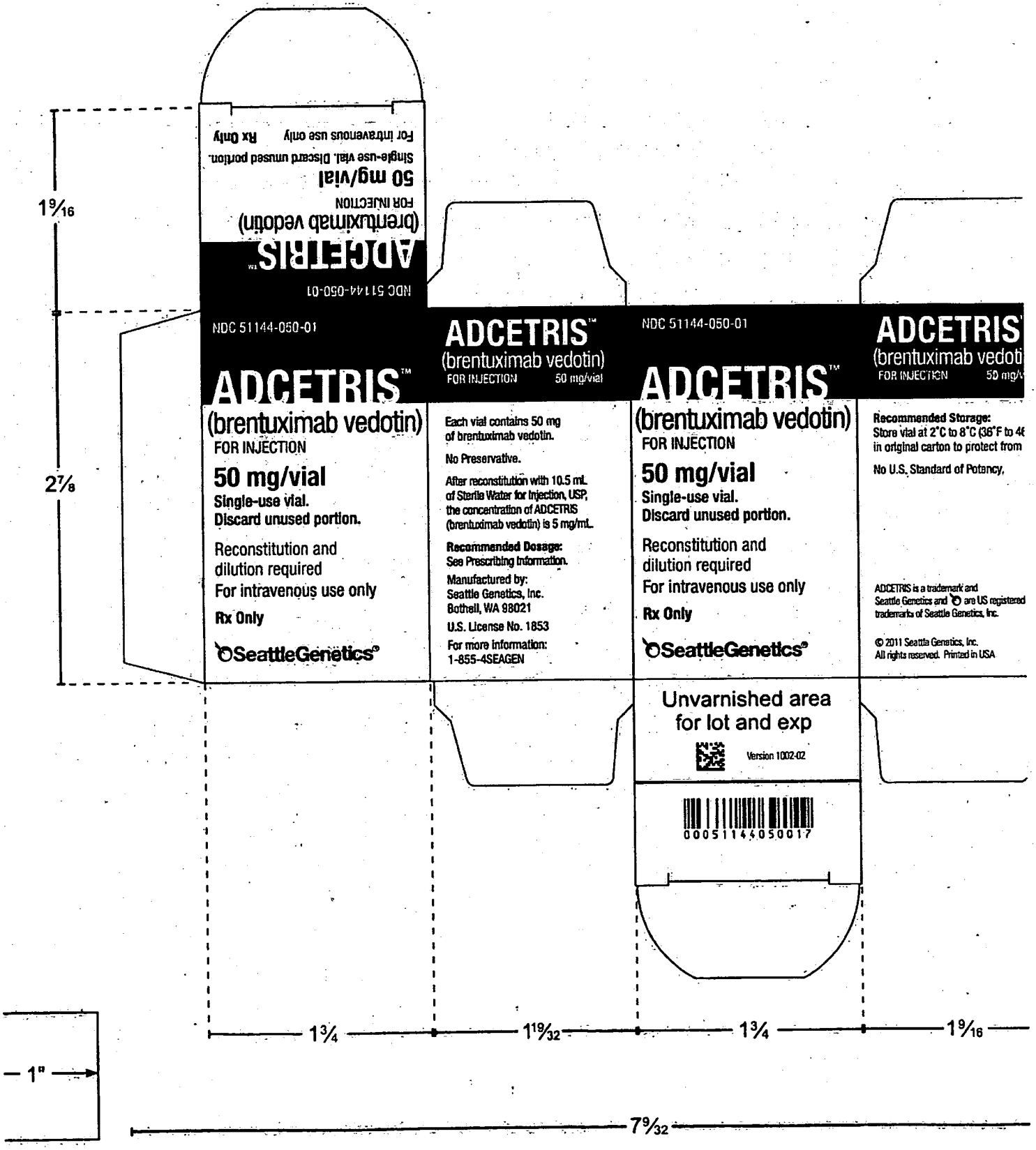
• **Pregnancy and Nursing**

ADCETRIS can cause fetal harm. Advise women receiving ADCETRIS to avoid pregnancy. Advise patients to report pregnancy immediately *[see Warnings and Precautions (5.7)]*. Advise patients to avoid nursing while receiving ADCETRIS *[see Use in Specific Populations (8.3)]*.

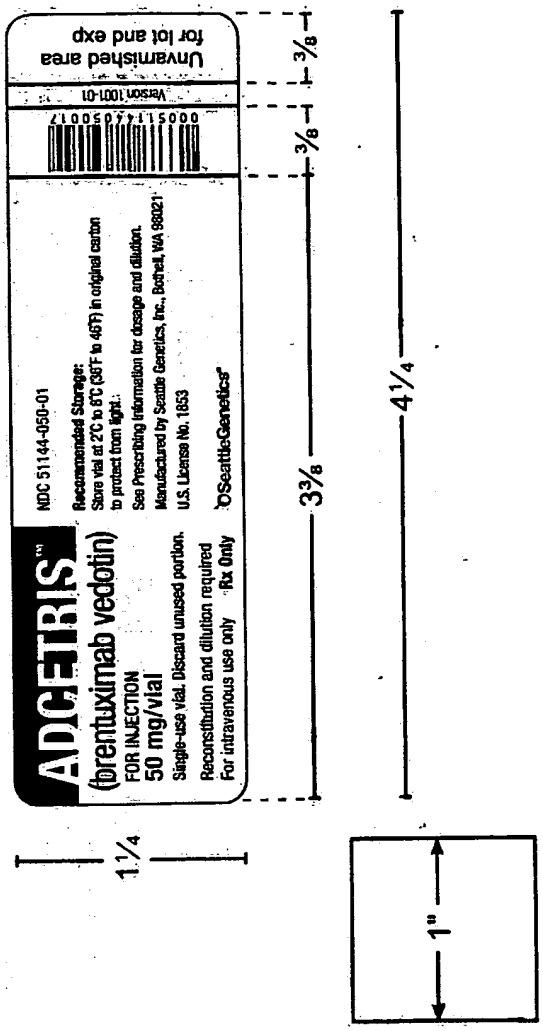
SeattleGenetics®

Manufactured by:
Seattle Genetics, Inc.
Bothell, WA 98021
1-855-473-2436
U.S. License 1853

August 2011



: The Seattle Genetics logo only has one ® mark per client request.



HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use ADCETRIS safely and effectively. See full prescribing information for ADCETRIS.

ADCETRIS™ (brentuximab vedotin) for Injection

For intravenous infusion

Initial U.S. approval: 2011

INDICATIONS AND USAGE

ADCETRIS is a CD30-directed antibody-drug conjugate indicated for:

- The treatment of patients with Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates (1.1).
- The treatment of patients with systemic anaplastic large cell lymphoma after failure of at least one prior multi-agent chemotherapy regimen (1.2).

These indications are based on response rate. There are no data available demonstrating improvement in patient reported outcomes or survival with ADCETRIS.

DOSAGE AND ADMINISTRATION

- The recommended dose is 1.8 mg/kg administered only as an intravenous infusion over 30 minutes every 3 weeks (2).
- Continue treatment until a maximum of 16 cycles, disease progression or unacceptable toxicity.

DOSAGE FORMS AND STRENGTHS

50 mg single-use vial (3).

CONTRAINDICATIONS

None (4).

WARNINGS AND PRECAUTIONS

- Peripheral neuropathy: Treating physicians should monitor patients for neuropathy and institute dose modifications accordingly (5.1).
- Infusion reactions: If an infusion reaction occurs, the infusion should be interrupted and appropriate medical management instituted. If anaphylaxis occurs, the infusion should be discontinued immediately and appropriate medical management instituted (5.2).
- Neutropenia: Monitor complete blood counts prior to each dose of ADCETRIS. If Grade 3 or 4 neutropenia develops, manage by dose delays, reductions or discontinuation (5.3).
- Tumor Lysis Syndrome: Patients with rapidly proliferating tumor and high tumor burden are at risk of tumor lysis syndrome and these patients should be monitored closely and appropriate measures taken (5.4).
- Stevens-Johnson syndrome: If Stevens-Johnson syndrome occurs, discontinue ADCETRIS and administer appropriate medical therapy (5.5).
- Progressive Multifocal Leukoencephalopathy (PML): A fatal case of PML has been reported in a patient who received 4 chemotherapy regimens prior to receiving ADCETRIS (5.6).
- Use in pregnancy: Fetal harm can occur. Pregnant women should be advised of the potential hazard to the fetus (5.7).

ADVERSE REACTIONS

The most common adverse reactions (≥20%) are neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia, cough, and vomiting (6.1).

To report SUSPECTED ADVERSE REACTIONS, contact Seattle Genetics, Inc. at 1-855-473-2436 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Patients who are receiving strong CYP3A4 inhibitors concomitantly with ADCETRIS should be closely monitored for adverse reactions (7.1).

USE IN SPECIFIC POPULATIONS

None (8).

See 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS

1 INDICATIONS AND USAGE
- 1.1 Hodgkin Lymphoma
- 1.2 Systemic Anaplastic Large Cell Lymphoma

2 DOSAGE AND ADMINISTRATION
- 2.1 General Dosing Information
- 2.2 Dose Modification
- 2.3 Instructions for Preparation and Administration

3 DOSAGE FORMS AND STRENGTHS

4 CONTRAINDICATIONS

5 WARNINGS AND PRECAUTIONS
- 5.1 Peripheral Neuropathy
- 5.2 Infusion Reactions
- 5.3 Neutropenia
- 5.4 Tumor Lysis Syndrome
- 5.5 Stevens-Johnson Syndrome
- 5.6 Progressive Multifocal Leukoencephalopathy
- 5.7 Use in Pregnancy

6 ADVERSE REACTIONS
- 6.1 Clinical Trial Experience
- 6.2 Immunogenicity

7 DRUG INTERACTIONS
- 7.1 Effect of Other Drugs on ADCETRIS
- 7.2 Effect of ADCETRIS on Other Drugs

8 USE IN SPECIFIC POPULATIONS
- 8.1 Pregnancy
- 8.3 Nursing Mothers
- 8.4 Pediatric Use
- 8.5 Geriatric Use
- 8.6 Renal Impairment
- 8.7 Hepatic Impairment

10 OVERDOSAGE

11 DESCRIPTION

12 CLINICAL PHARMACOLOGY
- 12.1 Mechanism of Action
- 12.2 Pharmacodynamics
- 12.3 Pharmacokinetics

13 NONCLINICAL TOXICOLOGY
- 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

14 CLINICAL STUDIES
- 14.1 Hodgkin Lymphoma
- 14.2 Systemic Anaplastic Large Cell Lymphoma

15 REFERENCES

16 HOW SUPPLIED/STORAGE AND HANDLING
- 16.1 How Supplied
- 16.2 Storage
- 16.3 Special Handling

17 PATIENT COUNSELING INFORMATION

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

These indications are based on response rate. There are no data available demonstrating improvement in patient reported outcomes or survival with ADCETRIS.

1.1 Hodgkin Lymphoma

ADCETRIS (brentuximab vedotin) is indicated for treatment of patients with Hodgkin lymphoma (HL) after failure of autologous stem cell transplant (ASCT) or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates.

1.2 Systemic Anaplastic Large Cell Lymphoma

ADCETRIS is indicated for treatment of patients with systemic anaplastic large cell lymphoma (sALCL) after failure of at least one prior multi-agent chemotherapy regimen.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

The recommended dose is 1.8 mg/kg administered only as an intravenous infusion over 30 minutes every 3 weeks.

Do not administer as an intravenous push or bolus.

Continue treatment until a maximum of 16 cycles, disease progression or unacceptable toxicity.

2.2 Dose Modification

Peripheral Neuropathy: Peripheral neuropathy should be managed using a combination of dose delay and reduction to 1.2 mg/kg. For new or worsening Grade 2 or 3 neuropathy, dosing should be held until neuropathy improves to Grade 1 or baseline and then restarted at 1.2 mg/kg. For Grade 4 peripheral neuropathy, ADCETRIS should be discontinued.

Neutropenia: Neutropenia should be managed by dose delays and reductions. The dose of ADCETRIS should be held for Grade 3 or 4 neutropenia until resolution to baseline or Grade 2 or lower. Growth factor support should be considered for subsequent cycles in patients who experience Grade 3 or 4 neutropenia. In patients with recurrent Grade 4 neutropenia despite the use of growth factors, discontinuation or dose reduction of ADCETRIS to 1.2 mg/kg may be considered.

2.3 Instructions for Preparation and Administration

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published [*see References (15)*].

Use appropriate aseptic technique for reconstitution and preparation of dosing solutions.

Reconstitution

Calculate the dose (mg) and number of vials of ADCETRIS required. The dose for patients with a weight of >100 kg should be calculated for 100 kg. Reconstitute each 50 mg vial of ADCETRIS with 10.5 mL of Sterile Water for Injection, USP, to yield a single-use solution containing 5 mg/mL brentuximab vedotin. Direct the stream toward wall of vial and not directly at the cake or powder. Gently swirl the vial to aid dissolution. **DO NOT SHAKE**. Inspect the reconstituted solution for particulates and discoloration. The reconstituted solution should be clear to slightly opalescent, colorless, and free of visible particulates. Following reconstitution, dilute immediately into an infusion bag, or store the solution at 2-8°C (36-46°F) and use within 24 hours of reconstitution. **DO NOT FREEZE.** Discard any unused portion left in the vial.

Dilution

Calculate the required volume of 5 mg/mL reconstituted ADCETRIS solution needed and withdraw this amount from the vials. The dose for patients with a weight of >100 kg should be calculated for 100 kg. Immediately add the reconstituted solution to an infusion bag containing a minimum volume of 100 mL to achieve a final concentration of 0.4 mg/mL to 1.8 mg/mL brentuximab vedotin. ADCETRIS can be diluted into 0.9% Sodium Chloride Injection, 5% Dextrose Injection or Lactated Ringer's Injection. Gently invert the bag to mix the solution. ADCETRIS contains no bacteriostatic preservatives. Following dilution, infuse the ADCETRIS solution immediately, or store the solution at 2-8°C (36-46°F) and use within 24 hours of reconstitution. **DO NOT FREEZE.**

Do not mix ADCETRIS with, or administer as an infusion with, other medicinal products.

3 DOSAGE FORMS AND STRENGTHS

ADCETRIS (brentuximab vedotin) for Injection single-use vial containing 50 mg of brentuximab vedotin as a sterile, white to off-white lyophilized, preservative-free cake or powder.

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Peripheral Neuropathy

ADCETRIS treatment causes a peripheral neuropathy that is predominantly sensory. Cases of peripheral motor neuropathy have also been reported. ADCETRIS-induced peripheral neuropathy is cumulative. In the HL and sALCL clinical trials, 54% of patients experienced any grade of neuropathy. Of these patients, 49% had complete resolution, 31% had partial improvement, and 20% had no improvement. Of the patients who reported neuropathy, 51% had residual neuropathy at the time of their last evaluation. Monitor patients for symptoms of neuropathy, such as hypoesthesia, hyperesthesia, paresthesia, discomfort, a burning sensation, neuropathic pain or weakness. Patients experiencing new or worsening peripheral neuropathy may require a delay, change in dose, or discontinuation of ADCETRIS [see *Dose Modification (2.2)*].

5.2 Infusion Reactions

Infusion-related reactions, including anaphylaxis, have occurred with ADCETRIS. Monitor patients during infusion. If anaphylaxis occurs, immediately and permanently discontinue administration of ADCETRIS and administer appropriate medical therapy. If an infusion-related reaction occurs, the infusion should be interrupted and appropriate medical management instituted. Patients who have experienced a prior infusion-related reaction should be premedicated for subsequent infusions. Premedication may include acetaminophen, an antihistamine and a corticosteroid.

5.3 Neutropenia

Complete blood counts should be monitored prior to each dose of ADCETRIS and more frequent monitoring should be considered for patients with Grade 3 or 4 neutropenia. Prolonged (≥1 week) severe neutropenia can occur with ADCETRIS. If Grade 3 or 4 neutropenia develops, manage by dose delays, reductions, or discontinuations [*see Dose Modification (2.2)*].

5.4 Tumor Lysis Syndrome

Tumor lysis syndrome may occur. Patients with rapidly proliferating tumor and high tumor burden may be at increased risk of tumor lysis syndrome. Monitor closely and take appropriate measures.

5.5 Stevens-Johnson Syndrome

Stevens-Johnson syndrome has been reported with ADCETRIS. If Stevens-Johnson syndrome occurs, discontinue ADCETRIS and administer appropriate medical therapy.

5.6 Progressive Multifocal Leukoencephalopathy

A fatal case of progressive multifocal leukoencephalopathy (PML) has been reported in a patient who received 4 chemotherapy regimens prior to receiving ADCETRIS.

5.7 Use in Pregnancy

There are no adequate and well-controlled studies of ADCETRIS in pregnant women. However, based on its mechanism of action and findings in animals, ADCETRIS can cause fetal harm when administered to a pregnant woman. Brentuximab vedotin caused embryo-fetal toxicities, including significantly decreased embryo viability and fetal malformations, in animals at maternal exposures that were similar to human exposures at the recommended doses for patients with HL and sALCL. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving the drug, the patient should be apprised of the potential hazard to the fetus *[see Use in Specific Populations (8.1)]*.

6 ADVERSE REACTIONS

6.1 Clinical Trial Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

ADCETRIS was studied as monotherapy in 160 patients in two phase 2 trials. Across both trials, the most common adverse reactions (≥20%), regardless of causality, were neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia, cough and vomiting. The most common adverse reactions occurring in at least 10% of patients in either trial, regardless of causality, using the NCI Common Toxicity Criteria Version 3.0, are shown in Table 1.

Experience in Hodgkin Lymphoma

ADCETRIS was studied in 102 patients with HL in a single arm clinical trial in which the recommended starting dose and schedule was 1.8 mg/kg intravenously every 3 weeks. Median duration of treatment was 27 weeks (range, 3 to 56 weeks) [*see Clinical Studies (14)*].

The most common adverse reactions (≥20%), regardless of causality, were neutropenia, peripheral sensory neuropathy, fatigue, upper respiratory tract infection, nausea, diarrhea, anemia, pyrexia, thrombocytopenia, rash, abdominal pain, cough, and vomiting.

Experience in Systemic Anaplastic Large Cell Lymphoma

ADCETRIS was studied in 58 patients with sALCL in a single arm clinical trial in which the recommended starting dose and schedule was 1.8 mg/kg intravenously every 3 weeks. Median duration of treatment was 24 weeks (range, 3 to 56 weeks) [*see Clinical Studies (14)*].

The most common adverse reactions (≥20%), regardless of causality, were neutropenia, anemia, peripheral sensory neuropathy, fatigue, nausea, pyrexia, rash, diarrhea, and pain.

Combined Experience

Table 1: Most Commonly Reported (≥10%) Adverse Reactions

	HL Total N = 102 % of patients			sALCL Total N = 58 % of patients		
Adverse Reaction	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
Blood and lymphatic system disorders						
Neutropenia*	54	15	6	55	12	9
Anemia*	33	8	2	52	2	-
Thrombocytopenia*	28	7	2	16	5	5
Lymphadenopathy	11	-	-	10	-	-
Nervous system disorders						
Peripheral sensory neuropathy	52	8	-	53	10	-
Peripheral motor neuropathy	16	4	-	7	3	-
Headache	19	-	-	16	2	-
Dizziness	11	-	-	16	-	-

Adverse Reaction	HL Total N = 102 % of patients			sALCL Total N = 58 % of patients		
	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
General disorders and administration site conditions						
Fatigue	49	3	-	41	2	2
Pyrexia	29	2	-	38	2	-
Chills	13	-	-	12	-	-
Pain	7	-	-	28	-	5
Edema peripheral	4	-	-	16	-	-
Infections and infestations						
Upper respiratory tract infection	47	-	-	12	-	-
Gastrointestinal disorders						
Nausea	42	-	-	38	2	-
Diarrhea	36	1	-	29	3	-
Abdominal pain	25	2	1	9	2	-
Vomiting	22	-	-	17	3	-
Constipation	16	-	-	19	2	-
Skin and subcutaneous tissue disorders						
Rash	27	-	-	31	-	-
Pruritus	17	-	-	19	-	-
Alopecia	13	-	-	14	-	-
Night sweats	12	-	-	9	-	-
Dry skin	4	-	-	10	-	-
Respiratory, thoracic and mediastinal disorders						
Cough	25	-	-	17	-	-
Dyspnea	13	1	-	19	2	-
Oropharyngeal pain	11	-	-	9	-	-
Musculoskeletal and connective tissue disorders						
Arthralgia	19	-	-	9	-	-
Myalgia	17	-	-	16	2	-
Back pain	14	-	-	10	2	-
Pain in extremity	10	-	-	10	2	2
Muscle spasms	9	-	-	10	2	-
Psychiatric disorders						
Insomnia	14	-	-	16	-	-
Anxiety	11	2	-	7	-	-

	HL Total N = 102 % of patients			sALCL Total N = 58 % of patients		
Adverse Reaction	Any Grade	Grade 3	Grade 4	Any Grade	Grade 3	Grade 4
Metabolism and nutrition disorders						
Decreased appetite	11	-	-	16	2	-
Investigations						
Weight decreased	6	-	-	12	3	-

*Derived from laboratory values and adverse reaction data

Infusion reactions

Two cases of anaphylaxis were reported in phase 1 trials. There were no Grade 3 or 4 infusion-related reactions reported in the phase 2 trials, however, Grade 1 or 2 infusion-related reactions were reported for 19 patients (12%). The most common adverse reactions (≥2%) associated with infusion-related reactions were chills (4%), nausea (3%), dyspnea (3%), pruritus (3%), pyrexia (2%), and cough (2%).

Serious adverse reactions

In the phase 2 trials, serious adverse reactions, regardless of causality, were reported in 31% of patients receiving ADCETRIS. The most common serious adverse reactions experienced by patients with HL include peripheral motor neuropathy (4%), abdominal pain (3%), pulmonary embolism (2%), pneumonitis (2%), pneumothorax (2%), pyelonephritis (2%), and pyrexia (2%). The most common serious adverse reactions experienced by patients with sALCL were septic shock (3%), supraventricular arrhythmia (3%), pain in extremity (3%), and urinary tract infection (3%). Other important serious adverse reactions reported included one case each of PML, Stevens-Johnson syndrome and tumor lysis syndrome.

Dose modifications

Adverse reactions that led to dose delays in more than 5% of patients were neutropenia (14%) and peripheral sensory neuropathy (11%) [*see Dose Modification (2.2)*].

Discontinuations

Adverse reactions led to treatment discontinuation in 21% of patients. Adverse reactions that led to treatment discontinuation in 2 or more patients with HL or sALCL were peripheral sensory neuropathy (8%) and peripheral motor neuropathy (3%).

6.2 Immunogenicity

Patients with HL and sALCL in the phase 2 trials [*see Clinical Studies (14)*] were tested for antibodies to brentuximab vedotin every 3 weeks using a sensitive electrochemiluminescent immunoassay. Approximately 7% of patients in these trials developed persistently positive

antibodies (positive test at more than 2 timepoints) and 30% developed transiently positive antibodies (positive in 1 or 2 post-baseline timepoints). The anti-brentuximab antibodies were directed against the antibody component of brentuximab vedotin in all patients with transiently or persistently positive antibodies. Two of the patients (1%) with persistently positive antibodies experienced adverse reactions consistent with infusion reactions that led to discontinuation of treatment. Overall, a higher incidence of infusion related reactions was observed in patients who developed persistently positive antibodies.

A total of 58 patient samples that were either transiently or persistently positive for anti-brentuximab vedotin antibodies were tested for the presence of neutralizing antibodies. Sixty-two percent of these patients had at least one sample that was positive for the presence of neutralizing antibodies. The effect of anti-brentuximab vedotin antibodies on safety and efficacy is not known.

Immunogenicity assay results are highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to ADCETRIS with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

In vitro data indicate that monomethyl auristatin E (MMAE) is a substrate and an inhibitor of CYP3A4/5.

7.1 Effect of Other Drugs on ADCETRIS

CYP3A4 Inhibitors/Inducers: MMAE is primarily metabolized by CYP3A [*see Clinical Pharmacology (12.3)*]. Co-administration of ADCETRIS with ketoconazole, a potent CYP3A4 inhibitor, increased exposure to MMAE by approximately 34%. Patients who are receiving strong CYP3A4 inhibitors concomitantly with ADCETRIS should be closely monitored for adverse reactions. Co-administration of ADCETRIS with rifampin, a potent CYP3A4 inducer, reduced exposure to MMAE by approximately 46%.

7.2 Effect of ADCETRIS on Other Drugs

Co-administration of ADCETRIS did not affect exposure to midazolam, a CYP3A4 substrate. MMAE does not inhibit other CYP enzymes at relevant clinical concentrations [*see Clinical Pharmacology (12.3)*]. ADCETRIS is not expected to alter the exposure to drugs that are metabolized by CYP3A4 enzymes.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D *[see Warnings and Precautions (5.7)]*.

There are no adequate and well-controlled studies with ADCETRIS in pregnant women. However, based on its mechanism of action and findings in animals, ADCETRIS can cause fetal harm when administered to a pregnant woman. Brentuximab vedotin caused embryo-fetal

toxicities in animals at maternal exposures that were similar to human exposures at the recommended doses for patients with HL and sALCL. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus.

In an embryo-fetal developmental study, pregnant rats received 2 intravenous doses of 0.3, 1, 3, or 10 mg/kg brentuximab vedotin during the period of organogenesis (once each on Pregnancy Days 6 and 13). Drug-induced embryo-fetal toxicities were seen mainly in animals treated with 3 and 10 mg/kg of the drug and included increased early resorption (≥99%), post-implantation loss (≥99%), decreased numbers of live fetuses, and external malformations (i.e., umbilical hernias and malrotated hindlimbs). Systemic exposure in animals at the brentuximab vedotin dose of 3 mg/kg is approximately the same exposure in patients with HL or sALCL who received the recommended dose of 1.8 mg/kg every three weeks.

8.3 Nursing Mothers

It is not known whether brentuximab vedotin is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from ADCETRIS a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

The safety and effectiveness of ADCETRIS have not been established in the pediatric population. Clinical trials of ADCETRIS included only 9 pediatric patients and this number is not sufficient to determine whether they respond differently than adult patients.

8.5 Geriatric Use

Clinical trials of ADCETRIS did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. Safety and efficacy have not been established.

8.6 Renal Impairment

The kidney is a route of excretion for MMAE. The influence of renal impairment on the pharmacokinetics of MMAE has not been determined.

8.7 Hepatic Impairment

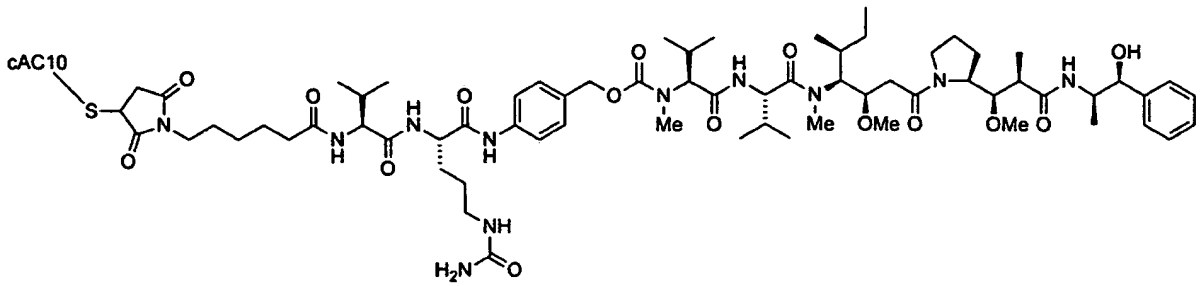
The liver is a route of clearance for MMAE. The influence of hepatic impairment on the pharmacokinetics of MMAE has not been determined.

10 OVERDOSAGE

There is no known antidote for overdosage of ADCETRIS. In case of overdosage, the patient should be closely monitored for adverse reactions, particularly neutropenia, and supportive treatment should be administered.

11 DESCRIPTION

ADCETRIS (brentuximab vedotin) is a CD30-directed antibody-drug conjugate (ADC) consisting of three components: 1) the chimeric IgG1 antibody cAC10, specific for human CD30, 2) the microtubule disrupting agent MMAE, and 3) a protease-cleavable linker that covalently attaches MMAE to cAC10.



Brentuximab vedotin has an approximate molecular weight of 153 kDa. Approximately 4 molecules of MMAE are attached to each antibody molecule. Brentuximab vedotin is produced by chemical conjugation of the antibody and small molecule components. The antibody is produced by mammalian (Chinese hamster ovary) cells, and the small molecule components are produced by chemical synthesis.

ADCETRIS (brentuximab vedotin) for Injection is supplied as a sterile, white to off-white, preservative-free lyophilized cake or powder in single-use vials. Following reconstitution with 10.5 mL Sterile Water for Injection, USP, a solution containing 5 mg/mL brentuximab vedotin is produced. The reconstituted product contains 70 mg/mL trehalose dihydrate, 5.6 mg/mL sodium citrate dihydrate, 0.21 mg/mL citric acid monohydrate, and 0.20 mg/mL polysorbate 80 and water for injection. The pH is approximately 6.6.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Brentuximab vedotin is an ADC. The antibody is a chimeric IgG1 directed against CD30. The small molecule, MMAE, is a microtubule disrupting agent. MMAE is covalently attached to the antibody via a linker. Nonclinical data suggest that the anticancer activity of ADCETRIS is due to the binding of the ADC to CD30-expressing cells, followed by internalization of the ADC-CD30 complex, and the release of MMAE via proteolytic cleavage. Binding of MMAE to tubulin disrupts the microtubule network within the cell, subsequently inducing cell cycle arrest and apoptotic death of the cells.

12.2 Pharmacodynamics

QT/QTc Prolongation Potential

The effect of brentuximab vedotin (1.8 mg/kg) on the QTc interval was evaluated in an open-label, single-arm study in 46 evaluable patients with CD30-expressing hematologic malignancies. Administration of brentuximab vedotin did not prolong the mean QTc interval

>10 ms from baseline. Small increases in the mean QTc interval (<10 ms) cannot be excluded because this study did not include a placebo arm and a positive control arm.

12.3 Pharmacokinetics

The pharmacokinetics of brentuximab vedotin were evaluated in phase 1 trials and in a population pharmacokinetic analysis of data from 314 patients. The pharmacokinetics of three analytes were determined: the ADC, MMAE, and total antibody. Total antibody had the greatest exposure and had a similar PK profile as the ADC. Hence, data on the PK of the ADC and MMAE have been summarized.

Absorption

Maximum concentrations of ADC were typically observed close to the end of infusion. A multiexponential decline in ADC serum concentrations was observed with a terminal half-life of approximately 4 to 6 days. Exposures were approximately dose proportional from 1.2 to 2.7 mg/kg. Steady-state of the ADC was achieved within 21 days with every 3-week dosing of ADCETRIS, consistent with the terminal half-life estimate. Minimal to no accumulation of ADC was observed with multiple doses at the every 3-week schedule.

The time to maximum concentration for MMAE ranged from approximately 1 to 3 days. Similar to the ADC, steady-state of MMAE was achieved within 21 days with every 3 week dosing of ADCETRIS. MMAE exposures decreased with continued administration of ADCETRIS with approximately 50% to 80% of the exposure of the first dose being observed at subsequent doses.

Distribution

In vitro, the binding of MMAE to human plasma proteins ranged from 68-82%. MMAE is not likely to displace or to be displaced by highly protein-bound drugs. In vitro, MMAE was a substrate of P-gp and was not a potent inhibitor of P-gp.

In humans, the mean steady state volume of distribution was approximately 6-10 L for ADC.

Metabolism

In vivo data in animals and humans suggest that only a small fraction of MMAE released from brentuximab vedotin is metabolized. In vitro data indicate that the MMAE metabolism that occurs is primarily via oxidation by CYP3A4/5. In vitro studies using human liver microsomes indicate that MMAE inhibits CYP3A4/5 but not other CYP isoforms. MMAE did not induce any major CYP450 enzymes in primary cultures of human hepatocytes.

Elimination

MMAE appeared to follow metabolite kinetics, with the elimination of MMAE appearing to be limited by its rate of release from ADC. An excretion study was undertaken in patients who received a dose of 1.8 mg/kg of ADCETRIS. Approximately 24% of the total MMAE administered as part of the ADC during an ADCETRIS infusion was recovered in both urine and feces over a 1-week period. Of the recovered MMAE, approximately 72% was recovered in the feces and the majority of the excreted MMAE was unchanged.

Effects of Gender, Age and Race

Based on the population pharmacokinetic analysis, gender, age and race do not have a meaningful effect on the pharmacokinetics of brentuximab vedotin.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies with brentuximab vedotin or the small molecule (MMAE) have not been conducted.

MMAE was genotoxic in the rat bone marrow micronucleus study through an aneugenic mechanism. This effect is consistent with the pharmacological effect of MMAE as a microtubule disrupting agent. MMAE was not mutagenic in the bacterial reverse mutation assay (Ames test) or the L5178Y mouse lymphoma forward mutation assay.

Fertility studies with brentuximab vedotin or MMAE have not been conducted. However, results of repeat-dose toxicity studies in rats indicate the potential for brentuximab vedotin to impair male reproductive function and fertility. In a 4-week repeat-dose toxicity study in rats with weekly dosing at 0.5, 5 or 10 mg/kg brentuximab vedotin, seminiferous tubule degeneration, Sertoli cell vacuolation, reduced spermatogenesis and aspermia were observed. Effects in animals were seen mainly at 5 and 10 mg/kg of brentuximab vedotin. These doses are approximately 3 and 6-fold the human recommended dose of 1.8 mg/kg, respectively, based on body weight.

14 CLINICAL STUDIES

14.1 Hodgkin Lymphoma

The efficacy of ADCETRIS in patients with HL who relapsed after autologous stem cell transplant was evaluated in one open-label, single-arm, multicenter trial. One hundred two patients were treated with 1.8 mg/kg of ADCETRIS intravenously over 30 minutes every 3 weeks. An independent review facility performed efficacy evaluations which included overall response rate (ORR = complete remission [CR] + partial remission [PR]) and duration of response as defined by clinical and radiographic measures including computed tomography (CT) and positron-emission tomography (PET) as defined in the 2007 Revised Response Criteria for Malignant Lymphoma (modified).

The 102 patients ranged in age from 15-77 years (median, 31 years) and most were female (53%) and white (87%). Patients had received a median of 5 prior therapies including autologous stem cell transplant.

The efficacy results are summarized in Table 2. Duration of response is calculated from date of first response to date of progression or data cutoff date.

Table 2: Efficacy Results in Patients with Hodgkin Lymphoma

	N=102		
	Percent (95%CI)	Duration of Response, in months	
		Median (95% CI)	Range
CR	32 (23, 42)	20.5 (12.0, NE*)	1.4 to 21.9+
PR	40 (32, 49)	3.5 (2.2, 4.1)	1.3 to 18.7
ORR	73 (65, 83)	6.7 (4.0, 14.8)	1.3 to 21.9+

*Not estimable

+ Follow up was ongoing at the time of data submission.

14.2 Systemic Anaplastic Large Cell Lymphoma

The efficacy of ADCETRIS in patients with relapsed sALCL was evaluated in one phase 2 open-label, single-arm, multicenter trial. This trial included patients who had sALCL that was relapsed after prior therapy. Fifty-eight patients were treated with 1.8 mg/kg of ADCETRIS administered intravenously over 30 minutes every 3 weeks. An independent review facility performed efficacy evaluations which included overall response rate (ORR = complete remission [CR] + partial remission [PR]) and duration of response as defined by clinical and radiographic measures including computed tomography (CT) and positron-emission tomography (PET) as defined in the 2007 Revised Response Criteria for Malignant Lymphoma (modified).

The 58 patients ranged in age from 14-76 years (median, 52 years) and most were male (57%) and white (83%). Patients had received a median of 2 prior therapies; 26% of patients had received prior autologous stem cell transplant. Fifty percent (50%) of patients were relapsed and 50% of patients were refractory to their most recent prior therapy. Seventy-two percent (72%) were anaplastic lymphoma kinase (ALK)-negative.

The efficacy results are summarized in Table 3. Duration of response is calculated from date of first response to date of progression or data cutoff date.

Table 3: Efficacy Results in Patients with Systemic Anaplastic Large Cell Lymphoma

	N=58		
	Percent (95%CI)	Duration of Response, in months	
		Median (95% CI)	Range
CR	57 (44, 70)	13.2 (10.8, NE*)	0.7 to 15.9+
PR	29 (18, 41)	2.1 (1.3, 5.7)	0.1 to 15.8+
ORR	86 (77, 95)	12.6 (5.7, NE*)	0.1 to 15.9+

*Not estimable

+ Follow up was ongoing at the time of data submission.

15 REFERENCES

1. NIOSH Alert: Preventing occupational exposure to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165
2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. Am J Health-Syst Pharm. (2006) 63:1172-1193
4. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

ADCETRIS (brentuximab vedotin) for Injection is supplied as a sterile, white to off-white preservative-free lyophilized cake or powder in individually-boxed single-use vials:

- NDC (51144-050-01), 50 mg brentuximab vedotin.

16.2 Storage

Store vial at 2-8°C (36-46°F) in the original carton to protect from light.

16.3 Special Handling

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published[1-4].

17 PATIENT COUNSELING INFORMATION

• Peripheral neuropathy

Advise patients that ADCETRIS can cause a peripheral neuropathy. They should be advised to report to their health care provider any numbness or tingling of the hands or feet or any muscle weakness [*see Warnings and Precautions (5.1)*].

• Fever/Neutropenia

Advise patients to contact their health care provider if a fever of 100.5°F or greater or other evidence of potential infection such as chills, cough, or pain on urination develops [*see Warnings and Precautions (5.3)*].

• Infusion reactions

Advise patients to contact their health care provider if they experience signs and symptoms of infusion reactions including fever, chills, rash, or breathing problems within 24 hours of infusion [*see Warnings and Precautions (5.2)*].

• Pregnancy and Nursing

ADCETRIS can cause fetal harm. Advise women receiving ADCETRIS to avoid pregnancy. Advise patients to report pregnancy immediately *[see Warnings and Precautions (5.7)]*. Advise patients to avoid nursing while receiving ADCETRIS *[see Use in Specific Populations (8.3)]*.

SeattleGenetics®

Manufactured by:
Seattle Genetics, Inc.
Bothell, WA 98021
1-855-473-2436
U.S. License 1853

August 2011

US007090843B1

(12) **United States Patent**
Francisco et al.

(10) **Patent No.: US 7,090,843 B1**
(45) **Date of Patent: Aug. 15, 2006**

(54) **RECOMBINANT ANTI-CD30 ANTIBODIES AND USES THEREOF**

(75) Inventors: **Joseph A. Francisco**, Edmonds, WA (US); **Grant Risdon**, Clayton, MO (US); **Alan F. Wahl**, Mercer Island, WA (US); **Clay Siegall**, Edmonds, WA (US)

(73) Assignee: **Seattle Genetics, Inc.**, Bothell, WA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/724,406**

(22) Filed: **Nov. 28, 2000**

(51) **Int. Cl.**
A61K 39/395 (2006.01)

(52) **U.S. Cl.** **424/138.1**; 424/141.1; 424/155.1; 424/134.1; 424/133.1

(58) **Field of Classification Search** 424/193.1, 424/138.1, 141.1, 155.1, 134.1, 133.1; 435/7.1; 530/387.3; 514/2
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,165,923	A *	11/1992	Thorpe et al.	
5,677,430	A	10/1997	Goodwin et al.	
5,789,554	A *	8/1998	Leung et al.	435/6
5,866,372	A	2/1999	Stein et al.	
6,033,876	A	3/2000	Lemke et al.	
2003/0186384	A1	10/2003	Barth et al.	
2004/0018194	A1	1/2004	Francisco et al.	
2005/0123536	A1	6/2005	Law et al.	

FOREIGN PATENT DOCUMENTS

DE	19543039	C1	11/1996
EP	0 678 523	A	10/1995
WO	WO 91/07437	A2	5/1991
WO	WO 96/22384	*	7/1996
WO	WO 97/17374		5/1997
WO	WO 01/80880	A2	11/2001
WO	WO 05/001038	A2	1/2005

OTHER PUBLICATIONS

Engert, et al., 1999, Seminars in Hematology, 36(3)282-289.*
da Costa, et al., 2000, Cancer Chemother Pharmacol, 46(suppl):S33-S36.*
Barth et al (Jun. 2000, Blood, vol. 95, p. 3909-14).*
Weigert et al (Nature Dec. 21-28, 1978;276:785-90, abstract).*
Falini et al., 1995, Blood, vol. 85, pp. 1-14.*
Tian et al., Cancer Res. Nov. 15, 1995; 55(22): 5335-41.*
Voet et al., (1990, Biochemistry, John Wiley & Sons, p. 1099-1101 only).*
Arndt et al., 1999, "A Bispecific Diabody That Mediates Natural Killer Cell Cytotoxicity Against Xenotransplantated Human Hodgkin's Tumors," Blood 94(8):2562-2568.
Barth et al., 2000, "Ki-4(scFv)-ETA', a new recombinant anti-CD30 immunotoxin with highly specific cytotoxic activity against disseminated Hodgkin tumors in SCID mice," Blood 95:3909-3914.
Bowen et al., 1993, "Functional Effects of CD30 on a Large Granular Lymphoma Cell Line, YT," J. Immunol. 151:5896-5906.
Cerutti et al., 1998, "CD30 Is a CD40-Inducible Molecule that Negatively Regulates CD40-Mediated Immunoglobulin Class Switching in Non-Antigen-Selected Human B Cells," Immunity 9:247-256.
Del Prete et al., 1995, "CD30-mediated Signaling Promotes the Development of Human T Helper Type 2-like T Cells," J. Exp. Med. 182:1655-1661.
Dürkop et al., 1992, "Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease.," Cell 68:421-427.
Engert et al., 1990, "Evaluation of Ricin A Chain-containing Immunotoxins Directed against the CD30 Antigen as Potential Reagents for the Treatment of Hodgkin's Disease," Cancer Res. 50:84-88.
Engert et al., 1999, "Treatment of Advanced Hodgkin's Lymphoma: Standard and Experimental Approaches," Seminars on Hematology 36(3):282-289.
Falini et al., 1992, "*In vivo* targeting of Hodgkin and Reed-Sternberg cells of Hodgkin's disease with monoclonal antibody Ber-H2 (CD30): immunohistological evidence," British J. Haemat. 82:38-45.
Falini et al., 1992, "Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin," The Lancet 339:1195-1196.
Franke et al., 2000, "Characterization of the CD30L Binding Domain on the Human CD30 Molecule Using Anti-CD30 Antibodies," Hybridoma 19:43-48.
Grell et al., 1999, "Induction of cell death by tumour necrosis factor (TNF) receptor 2, CD40 and CD30: a role for TNF-R1 activation by endogenous membrane-anchored TNF," EMBO J. 18(11):3034-3043.

(Continued)

Primary Examiner—Misook Yu
(74) *Attorney, Agent, or Firm*—Vita G. Conforti; Mark G. Sandbaken; Jones Day

(57) **ABSTRACT**

The present invention relates to methods and compositions for the treatment of Hodgkin's Disease, comprising administering proteins characterized by their ability to bind to CD30, or compete with monoclonal antibodies AC10 or HeFi-1 for binding to CD30, and exert a cytostatic or cytotoxic effect on Hodgkin's Disease cells. Such proteins include derivatives of monoclonal antibodies AC10 and HeFi-1. The proteins of the invention can be human, humanized, or chimeric antibodies; further, they can be conjugated to cytotoxic agents such as chemotherapeutic drugs. The invention further relates to nucleic acids encoding the proteins of the invention. The invention yet further relates to a method for identifying an anti-CD30 antibody useful for the treatment or prevention of Hodgkin's Disease.

18 Claims, 6 Drawing Sheets

OTHER PUBLICATIONS

Gruss et al., 1994, "Pleiotrophic Effects of the CD30 Ligand on CD30-Expressing Cells and Lymphoma Cell Lines," Blood 83(8):2045-2056.

Hansen et al., 1991, "Antibody-enhanced shedding of the CD30 (Ki-1) antigen and biochemical analysis of its soluble form," Immunobiol. 183:214.

Hecht et al., 1985, "Production and Characterization of a Monoclonal Antibody That Binds Reed-Sternber Cells," J. Immunol. 134(6):4231-4236.

Horn-Lohrens et al., 1995, "Shedding of the Soluble Form of CD30 From the Hodgkin-Analogose Cell Line L540 is Strongly Inhibited by a new CD30-Specific Antibody (Ki-4)," Int. J. Cancer 60:539-544.

Hsu et al., 1987, "Effect of Monoclonal Antibodies Anti-2H9, Anti-IRac, and Anti-HeFi-1 on the Surface Antigens of Reed-Sternberg Cells," JNCI 79(5):1091-1099.

Hübinger et al., 1999, "The tyrosine kinase NPM-ALK, associated with anaplastic large cell lymphoma, binds the intracellular domain of the suface receptor CD30 but is not activated by CD30 stimulation," Exp. Hematol. 27:1796-1805.

Josimovic-Alasevic et al., 1989, "Ki-1 (CD30) antigen is released by Ki-1-positive tumor cells in vitro and in vivo. I. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay," Eur. J. Immunol. 19:157-162.

Leca et al., 1994, "A Monoclonal Antibody to the Hodgkin's Disease-Associated Antigen CD30 Induces Activation and Long-Term Growth of Human Autoreactive γδ T Cell Clone," Cell. Immunol. 156:230-239.

Masuda et al., 1998, "Dual action of CD30 antigen: Anti-CD30 antibody induced apoptosis and interleukin-8 secretion in Ki-1 lymphoma cells," Int. J. Hematol. 67:257-265.

May et al., 1990, "Evaluation of ricin A chain-containing immunotoxins directed against different epitopes on the delta-chain of cell surface-associated IgD on murine B cells," J. Immunol. 144:3637-3642.

Mir et al., 2000, "Differential effects of CD30 activation in anaplastic large cell lymphoma and Hodgkin disease cells," Blood 96(13):4307-4312.

Nawrocki et al., "Biochemical and Structural Properties of a Hodgkin's Disease-Related Membrane Protein," J. Immunol. 141:672-680.

Pallesen, 1990, "The diagnostic significance of the CD30 (Ki-1) antigen," Histopatholgoy 16:409-413.

Powell et al., 1998, "Construction and expression of a soluble form of human CD30 ligand with functional activity," J. Leukocyte Biology 63:752-757.

Press et al., 1988, "Ricin A-chain containing immunotoxins directed against different epitopes on the CD2 molecule differ in their ability to kill normal and malignant T cells," J. Immunol. 141:4410-4417.

Renner et al., 2000, "Initiation of humoral and cellular immune responses in patients with refractory Hodgkin's disease by treatment with an anti-CD16/CD30 bispecific antibody," Cancer Immunol. Immunother. 49:173-180.

Schwab et al., 1982, "Production of a monoclonal antibody specific for Hodgkin and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells," Nature 299:65-67.

Schwarting et al., 1989, "BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formol-resistant epitope," Blood, Blood 74:1678-1689.

Smith et al., 1993, "CD30 Antigen, a Marker for Hodgkin's Lymphoma, Is a Receptor Whose Ligand Defines an Emerging Family of Cytokines with Homology to TNF," Cell 73:1349-1360.

Tian et al., "*in vivo* Antitiumor Effects of Unconjugated CD30 Monoclonal Antibodies of Human Anaplastic Large-Cell Lymphoma Xenografts," Cancer Res. 55:5335-5341.

Tsutsumi et al., 2000, "Site-specific chemical modification with polyethylene glycol of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) improves antitumor activity and reduces animal toxicity and immunogenicity," Proc. Natl. Acad. Sci, USA 97:8548-8553.

Tutt et al., 1998, "Monoclonal antibody therapy of B cell lymphoma: signaling activity on tumor cells appears more important than recruitment of effectors," J. Immunol. 161:3176-3185.

Hombach et al., 1998, "Generation of the single chain antibody fragment conserves the idiotypic profile of the anti-CD30 monoclonal antibody HRS3," Scand. J. Immunol. 48(5):497-501.

Schnell et al., 1995, Development of New Ricin A-Chain Immunotoxins with Potent Anti-Tumor Effects Against Human Hodgkin Cells in Vitro and Disseminated Hodgkin Tumors in SCID Mice, (1995).

Bauer al., 1999, "Immunotherapy of Human Tumors with T-Cell-activating Bispecific Antibodies: Stimulation of Cytotoxic Pathways *in Vivo*," Cancer Research, 59:1961-65.

Pohl et al., 1993, "CD30-Antigen-Specific Targeting and Activation of T Cells Via Murine Bispecific Monoclonal Antibodies Against CD3 and CD28: Potential Use for the Treatment of Hodgkin's Lymphoma," Int. J. Cancer 54:820-27.

Renner and Pfreundschuh, 1995, "Tumor Therapy by Immune Recruitment with Bispecific Antobodies," Immunoloy Reviews, 145:178-209.

Pohl et al., "CD30-Specific AB1-AB2-AB3 Internal Image Antibody Network: Potential Use as Anti-Idiotype Vaccine Against Hodgkin's Lymphoma," *Int. J. Cancer* 54:418-425 (1993).

Weigert et al., "Rearrangement of Genetic Information May Produce Immunoglobulin Diversity," *Nature* 276:785-790 (1978).

* cited by examiner

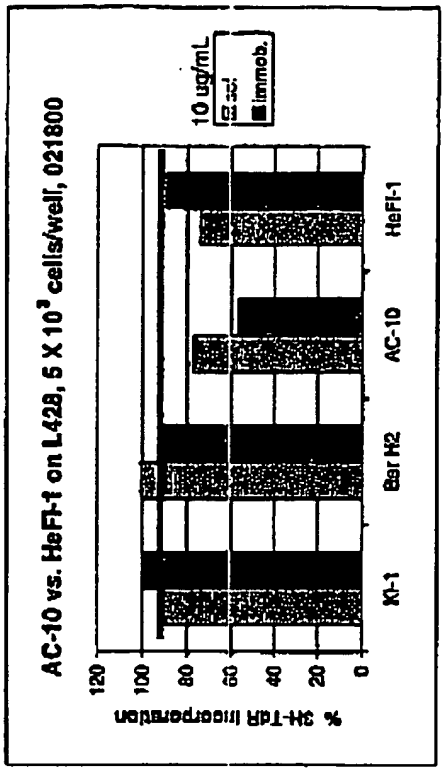


FIGURE 1b

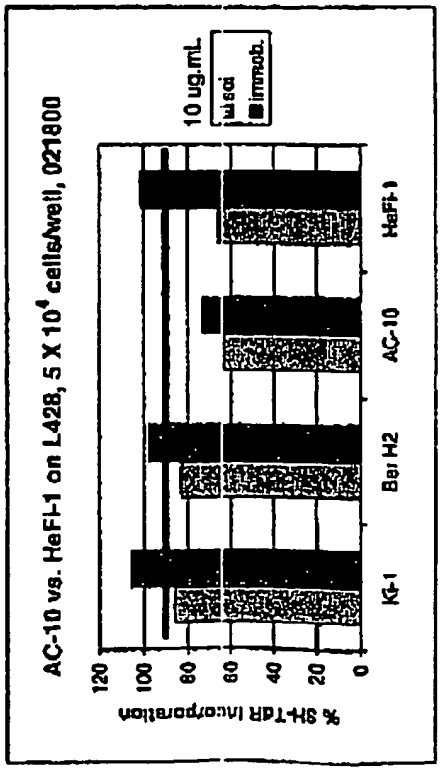


FIGURE 1a

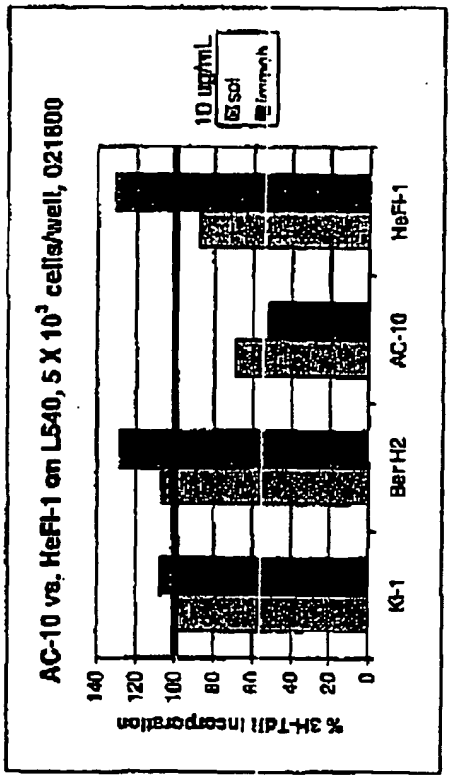


FIGURE 2b

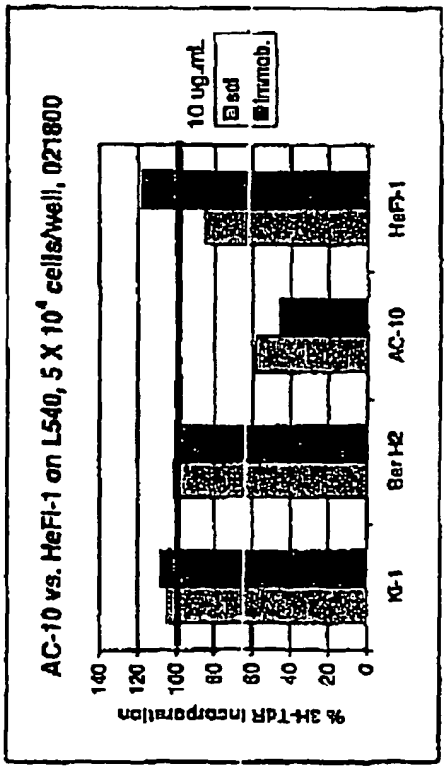


FIGURE 2a

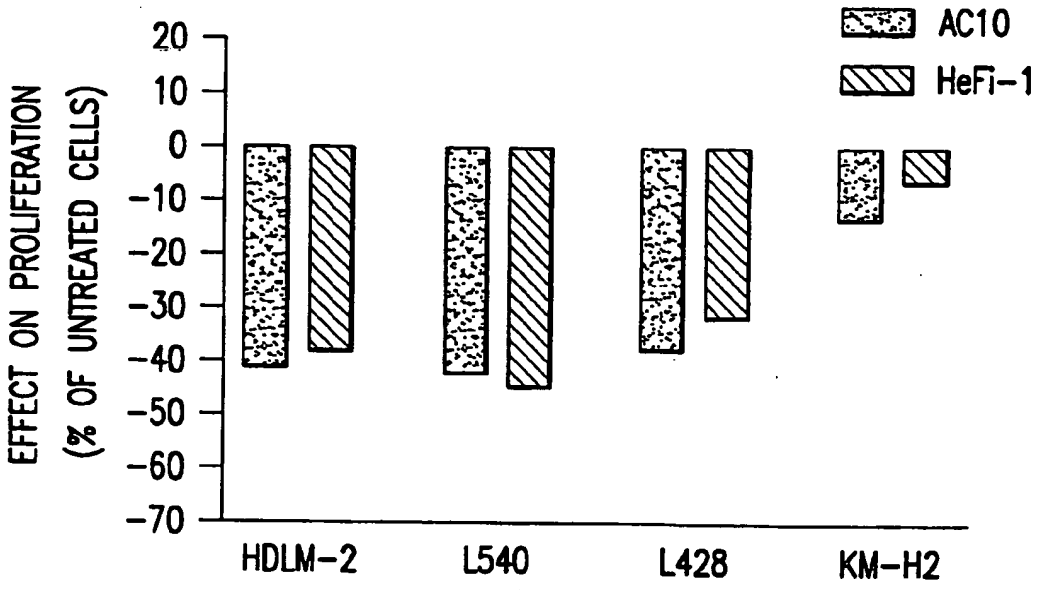

AC10
HeFi-1
EFFECT ON PROLIFERATION
(% OF UNTREATED CELLS)
20
10
0
-10
-20
-30
-40
-50
-60
-70
HDLM-2
L540
L428
KM-H2

FIG. 3

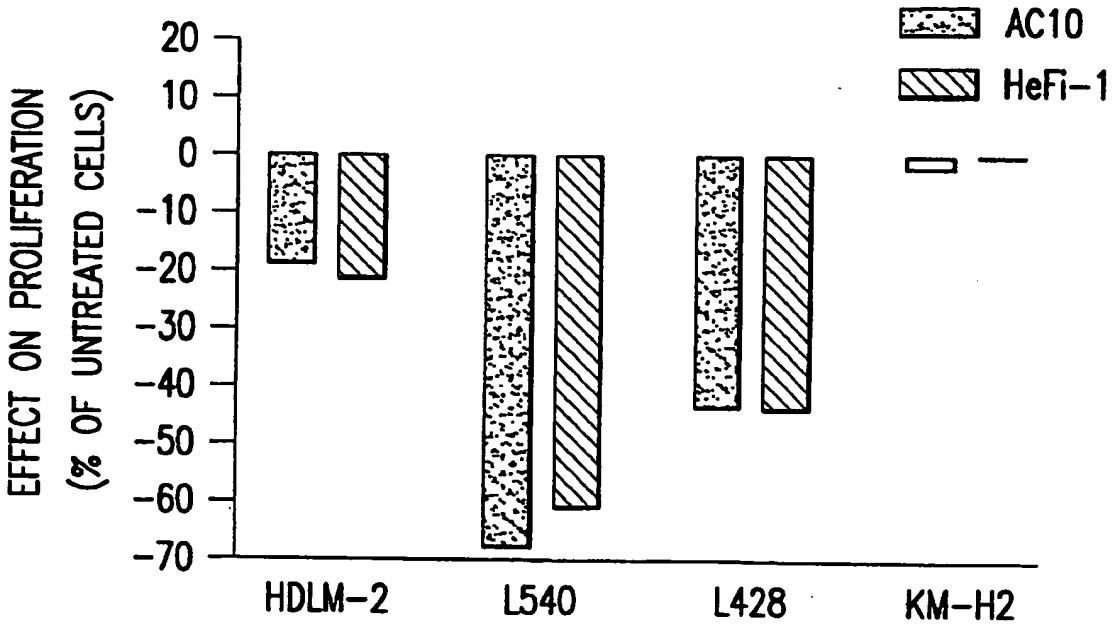

AC10
HeFi-1
EFFECT ON PROLIFERATION
(% OF UNTREATED CELLS)
20
10
0
-10
-20
-30
-40
-50
-60
-70
HDLM-2
L540
L428
KM-H2

FIG. 4

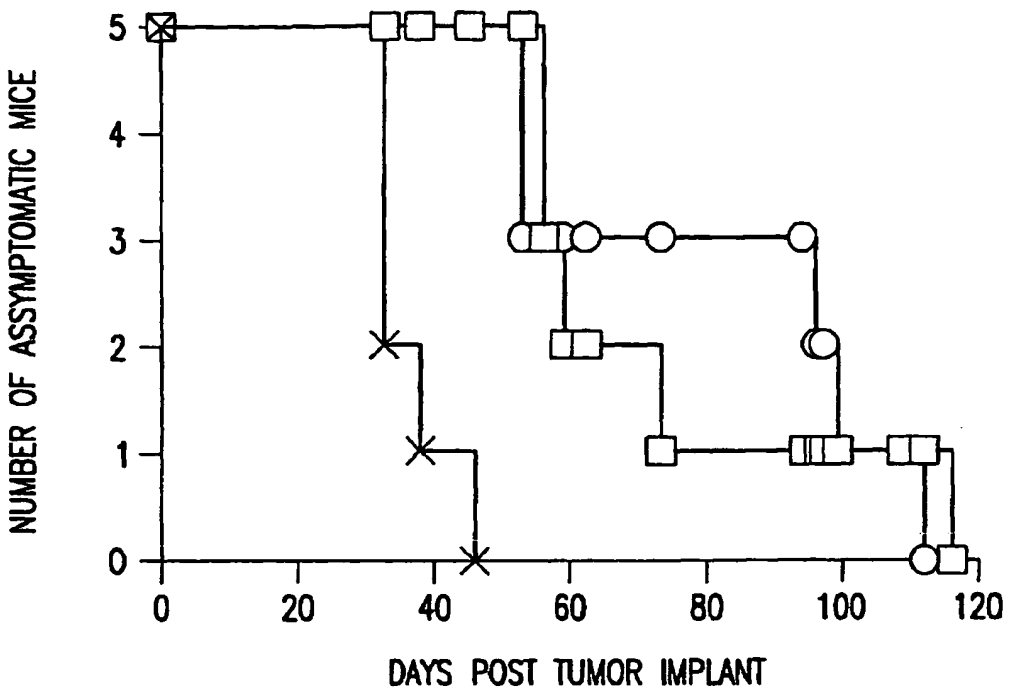

NUMBER OF ASSYMPTOMATIC MICE
0
1
2
3
4
5
0
20
40
60
80
100
120
DAYS POST TUMOR IMPLANT

FIG. 5A

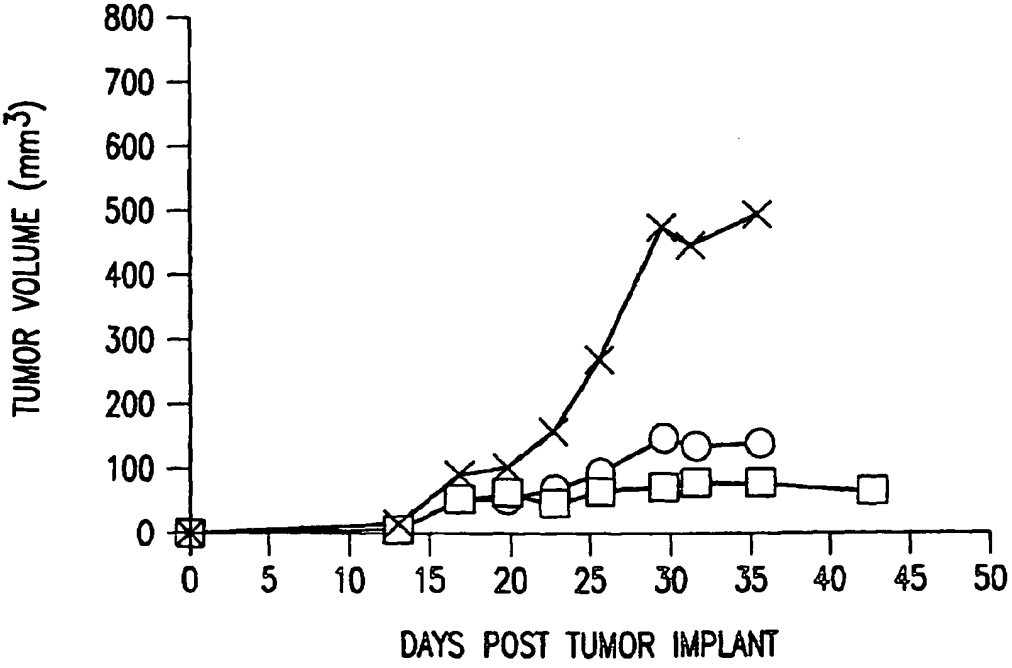

TUMOR VOLUME (mm3)
0
100
200
300
400
500
600
700
800
0
5
10
15
20
25
30
35
40
45
50
DAYS POST TUMOR IMPLANT

FIG. 5B

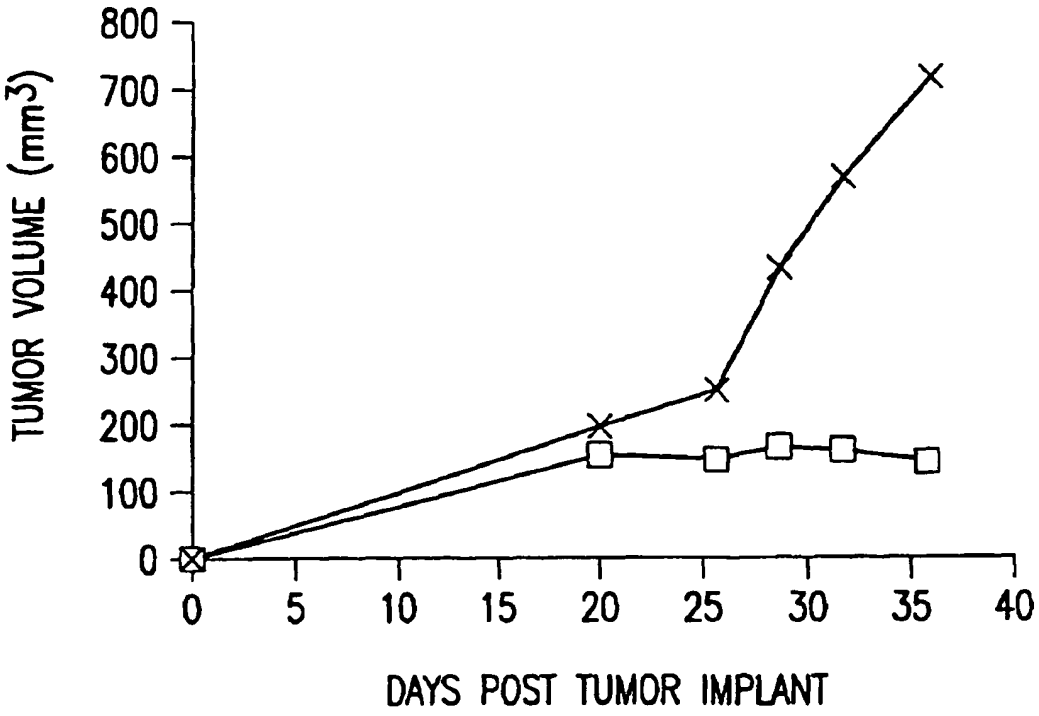

TUMOR VOLUME (mm3)
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
DAYS POST TUMOR IMPLANT

FIG. 6

RECOMBINANT ANTI-CD30 ANTIBODIES AND USES THEREOF

1. FIELD OF THE INVENTION

The present invention relates to methods and compositions for the treatment of Hodgkin's Disease, comprising administering a protein that binds to CD30. Such proteins include recombinant/variant forms of monoclonal antibodies AC10 and HeFi-1, and derivatives thereof. This invention relates to a novel class of monoclonal antibodies directed against the CD30 receptor which, in unmodified form, are capable of inhibiting the growth of CD30-expressing Hodgkin's Disease cells.

2. BACKGROUND OF THE INVENTION

Curative chemotherapy regimens for Hodgkin's disease represent one of the major breakthroughs in clinical oncology. Multi-agent chemotherapy regimens have increased the cure rate to more than 80% for these patients Nevertheless, 3% of patients die from treatment-related causes, and for patients who do not respond to standard therapy or relapse after first-line treatment, the only available treatment modality is high-dose chemotherapy in combination with stem cell transplantation. This treatment is associated with an 80% incidence of mortality, significant morbidity and a five-year survival rate of less than 50% (See e.g., Engert, et al., 1999, Seminars in Hematology 36:282–289).

The primary cause for tumor relapse is the development of tumor cell clones resistant to the chemotherapeutic agents. Immunotherapy represents an alterative strategy which can potentially bypass resistance. Monoclonal antibodies for specific targeting of malignant tumor cells has been the focus of a number of immunotherapeutic approaches. For several malignancies, antibody-based therapeutics are now an acknowledged part of the standard therapy. The engineered anti-CD20 antibody Rituxan®, for example, was approved in late 1997 for the treatment of relapsed low-grade NHL.

CD30 is a 120 kilodalton membrane glycoprotein (Froese et al., 1987, J. Immunol. 139: 2081–87) and a member of the TNF-receptor superfamily. This family includes TNF-RI, TNF-RII, CD30, CD40, OX-40 and RANK, among others.

CD30 is a proven marker of malignant cells in Hodgkin's disease (HD) and anaplastic large cell lymphoma (ALCL), a subset of non-Hodgkin's (NHL) lymphomas (Dürkop et al., 1992, Cell 88:421–427). Originally identified on cultured Hodgkin's-Reed Steinberg (H-RS) cells using the monoclonal antibody Ki-1 (Schwab et al., 1982, Nature 299: 65–67), CD30 is highly expressed on the cell surface of all HD lymphomas and the majority of ALCL, yet has very limited expression in normal tissues to small numbers of lymphoid cells in the perifollicular areas (Josimovic-Alasevic et al., 1989, Eur. J. Immunol. 19:157–162). Monoclonal antibodies specific for the CD30 antigen have been explored as vehicles for the delivery of cytostatic drugs, plant toxins and radioisotopes in both preclinical models and clinical studies (Engert et al., 1990, Cancer Research 50:84–88; Barth et al., 2000, Blood 95:3909–3914). In patients with HD, targeting of the CD30 antigen could be achieved with low doses of the anti-CD30 mAb, BerH2 (Falini et al., 1992, British Journal of Haematology 82:38–45). Yet, despite successful in vivo targeting of the malignant tumor cells, none of the patients experienced tumor regression. In a subsequent clinical trial, a toxin (saporin) was chemically conjugated to the antibody BerH2 and all four patients demonstrated rapid and substantial reductions in tumor mass (Falini et al., 1992, Lancet 339: 1195–1196).

These observations underscore the validity of the CD30 receptor as a target antigen. However, all of the patients treated with the mAb-toxin conjugate developed antibodies to the toxin. One of the major limitations of immunotoxins is their inherent immunogenicity that results in the development of antibodies to the toxin molecule and neutralizes their effects (Tsutsumi et al., 2000, Proc. Nat'l Acad. Sci. U.S.A. 97:8545–8553). Additionally, the liver toxicity and vascular leak syndrome associated with immunotoxins potentially limits the ability to deliver curative doses of these agents (Tsutsumi et al., 2000, Proc. Nat'l Acad. Sci. U.S.A. 97:8545–8553).

2.1 CD30 Monoclonal Antibodies

CD30 was originally identified by the monoclonal antibody Ki-1 and initially referred to as the Ki-1 antigen (Schwab et al., 1982, Nature 299:65–67). This mAb was developed against Hodgkin and Reed-Sternberg (H—RS) cells, the malignant cells of Hodgkin's disease (HD). A second mAb, capable of binding a formalin resistant epitope, different from that recognized by Ki-1 was subsequently described (Schwarting et al., 1989 Blood 74:1678–1689). The identification of four additional antibodies resulted in the creation of the CD30 cluster at the Third Leucocyte Typing Workshop in 1986 (McMichael, A., ed., 1987, Leukocyte Typing III (Oxford: Oxford University Press)).

2.2 CD30 Monoclonal Antibody-Based Therapeutics

The utility of CD30 mAbs in the diagnosis and staging of HD led to their evaluation as potential tools for immunotherapy. In patients with HD, specific targeting of the CD30 antigen was achieved with low doses (30–50 mg) of the anti-CD30 mAb BerH2 (Falini et al., 1992, British Journal of Haematology 82:38–45). Despite successful targeting in vivo of the malignant H—RS tumor cells, none of the patients experienced tumor regressions.

Based on these results, it was concluded that efficacy with CD30 mAb targeted immunotherapy could not be achieved with unmodified antibodies (Falini et al., 1992, Lancet 339:1195–1196). In a subsequent clinical trial, treatment of four patients with refractory HD with a toxin, saporin, chemically conjugated to the mAb BerH2 demonstrated rapid and substantial, although transient, reductions in tumor mass (Falini et al., 1992, Lancet 339:1195–1196). In recent years, investigators have worked to refine the approaches for treating CD30-expressing neoplastic cells. Examples include the development of recombinant single chain immunotoxins (Barth et al., 2000, Blood 95:3909–3914), anti-CD16/CD30 bi-specific mAbs (Renner et al., 2000, Cancer Immunol. Immunother. 49:173–180), and the identification of new anti-CD30 mAbs which prevent the release of CD30 molecules from the cell surface (Horn-Lohrens et al., 1995, Int. J. Cancer 60:539–544). This focus has dismissed the potential of anti-CD30 "mAbs with signaling activity in the treatment of Hodgkin's disease.

2.3. Identification of Anti-CD30 Monoclonal Antibodies with Agonist Activity

In cloning and characterizing the biologic activity of the human CD30 ligand (CD30L), two mAbs, M44 and M67, were described which mimicked the activity of CD30L induced receptor crosslinking (Gruss et al., 1994, Blood 83:2045–2056). In in vitro assays, these mAbs, in immobilized form, were capable of stimulating the proliferation of activated T-cells and the Hodgkin's disease cell lines of T-cell origin, L540 and HDLM-2. In contrast, these mAbs

had little effect on the Hodgkin's cell lines of B-cell origin, L428 and KM-H2 (Gruss et al., 1994, Blood 83:2045–2056). In all of these assays, the binding of the CD30 receptor by the anti-CD30 mAb Ki-1 had little effect.

The proliferative activity of these agonist anti-CD30 mAbs on Hodgkin's cell lines suggested that anti-CD30 mAbs possessing signaling activity would not have any utility in the treatment of HD.

In contrast, the proliferation of cell lines representing CD30-expressing ALCL was strongly inhibited by the presence of immobilized M44 and M67 mAbs. This inhibitory activity against ALCL cell lines was further extended to in vivo animal studies. The survival of SCID mice bearing ALCL tumor xenografts was significantly increased following the administration of the mAb M44. In addition, the anti-CD30 mAb HeFi-1, recognizing a similar epitope as that of M44, also prolonged survival in this animal model (Tian et al., 1995, Cancer Research 55:5335–5341).

There is a need in the art for therapeutics with increased efficacy to treat or prevent Hodgkin's Disease, a need provided by the present invention. Clinical trials and numerous pre-clinical evaluations have failed to demonstrate antitumor activity of a number of anti-CD30 mAbs in unmodified form against cells representative of Hodgkin's disease. Under conditions similar to those utilized by Gruss et al. in their evaluations of mAbs Ki-1, M44 and M67 (Gruss et al., 1994, Blood 83:2045–2056), we demonstrate a class of CD30 mAbs which is functionally distinct from those previously described. This class of anti-CD30 mAbs is capable of inhibiting the in vitro growth of all Hodgkin's lines tested. Furthermore, these unmodified mAbs possess in vivo antitumor activity against HD tumor xenografts.

2.3.1 Monoclonal Antibody AC10

The majority of murine anti-CD30 mAbs known in the art have been generated by immunization of mice with HD cell lines or purified CD30 antigen. AC10, originally termed C10 (Bowen et al., 1993, J. Immunol. 151:5896–5906), is distinct in that this anti-CD30 mAb that was prepared against a hum an NK-like cell line, YT (Bowen et al., 1993, J. Immunol. 151:5896–5906). Initially, the signaling activity of this mAb was evidenced by the down regulation of the cell surface expression of CD28 and CD45 molecules, the up regulation of cell surface CD25 expression and the induction of homotypic adhesion following binding of C10 to YT cells.

2.3.2 Monoclonal Antibody HeFi-1

HeFi-1 is an anti-CD30 mAb which was produced by immunizing mice with the L428 Hodgkin's disease cell line (Hecht et al., 1985, J. Immunol. 134:4231–4236). Coculture of HeFi-1 with the Hodgkin's disease cell lines L428 or L540 failed to reveal any direct effect of the mAb on the viability of these cell lines. In vitro and in vivo antitumor activity of HeFi-1 was described by Tian et al against the Karpas 299 ALCL cell line (Tian et al., 1995, Cancer Research 55:5335–5341).

2.4 Direct Anti-Tumor Activity of Signaling CD30 Antibodies

Monoclonal antibodies represent an attractive approach to targeting specific populations of cells in vivo. Native mAbs and their derivatives may eliminate tumor cells by a number of mechanisms including, but not limited to, complement activation, antibody dependent cellular cytotoxicity (ADCC), inhibition of cell cycle progression and induction of apoptosis (Tutt et al., 1998, J. Immunol. 161:3176–3185).

As described above, mAbs to the CD30 antigen such as Ki-1 and Ber-H2 failed to demonstrate direct antitumor activity (Falini et al., 1992, British Journal of Haematology 82:38–45; Gruss et al., 1994, Blood 83:2045–2056). While some signaling mAbs to CD30, including M44, M67 and HeFi-1, have been shown to inhibit the growth of ALCL lines in vitro (Gruss et al., 1994, Blood 83:2045–2056) or in vivo (Tian et al., 1995, Cancer Res. 55:5335–5341), known anti-CD30 antibodies have not been shown to be effective in inhibiting the proliferation of HD cells in culture. In fact, two signaling anti-CD30 mAbs, M44 and M67, which inhibited the growth of the ALCL line Karpas-299, were shown to enhance the proliferation of T-cell-like HD lines in vitro while showing no effect on B-cell-like HD lines (Gruss et al., 1994, Blood 83:2045–2056).

The conjugate of antibody Ki-1 with the Ricin A-chain made for a rather ineffective immunotoxin and it was concluded that this ineffectiveness was due to the rather low affinity of antibody Ki-1 (Engert et al., 1990, Cancer Research 50:84–88). Two other reasons may also account for the weak toxicity of Ki-1-Ricin A-chain conjugates: a) Antibody Ki-1 enhanced the release of the sCD30 from the Hodgkin-derived cell lines L428 and L540 as well as from the CD30+non-Hodgkin's lymphoma cell line Karpas 299 (Hansen et al., 1991, Immunobiol. 183:214); b) the relatively great distance of the Ki-1 epitope from the cell membrane is also not favorable for the construction of potent immunotoxins (Press et al., 1988, J. Immunol. 141: 4410–4417; May et al., 1990, J. Immunol. 144:3637–3642).

At the Fourth Workshop on Leukocyte Differentiation Antigens in Vienna in February 1989, monoclonal antibodies were submitted by three different laboratories and finally characterized as belonging to the CD30 group. Co-cultivation experiments by the inventors of L540 cells with various antibodies according to the state of the art, followed by the isolation of sCD30 from culture supernatant fluids, revealed that the release of the sCD30 was most strongly increased by antibody Ki-1, and weakly enhanced by the antibody HeFi-1, whilst being more strongly inhibited by the antibody Ber-H2. However, the antibody Ber-H2 also labels a subpopulation of plasma cells (Schwarting et al., 1988, Blood 74:1678–1689) and G. Pallesen (G. Pallesen, 1990, Histopathology 16:409–413) describes, on page 411, that Ber-H2 is cross-reacting with an epitope of an unrelated antigen which is altered by formaldehyde.

Citation or identification of any reference herein shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is based on the surprising discovery of a novel activity associated with a certain class of anti-CD30 antibodies, said class comprising AC10 and HeFi-1, namely their ability to inhibit the growth of both T-cell-like and B-cell-like Hodgkin's Disease (HD) cells.

The invention provides proteins that compete for binding to CD30 with monoclonal antibody AC10 or HeFi-1, and exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line. The invention further provides antibodies that immunospecifically bind CD30 and exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line.

The invention further provides a method for the treatment or prevention of Hodgkin's Disease in a subject comprising administering to the subject, in an amount effective for said treatment or prevention, an antibody that immunospecifically binds CD30 and exerts a cytostatic or cytotoxic effect

on a Hodgkin's Disease cell line; and a pharmaceutically acceptable carrier. The invention provides a method for the treatment or prevention of Hodgkin's Disease in a subject comprising administering to the subject an amount of a protein, which protein competes for binding to CD30 with monoclonal antibody AC10 or HeFi-1, and exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, which amount is effective for the treatment or prevention of Hodgkin's Disease. In one embodiment, a protein of the invention is conjugated to a cytotoxic molecule. In another embodiment, a protein of the invention is a fusion protein comprising the amino acid sequence of a second protein such as bryodin or a pro-drug converting enzyme. The proteins of the invention, including conjugates and fusion proteins, can be used in conjunction with radiation therapy, chemotherapy, hormonal therapy and/or immunotherapy.

In determining the cytostatic effect of the proteins of the invention on Hodgkin's Disease cell lines, a culture of the Hodgkin's Disease cell line is contacted with the protein, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours; exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72-hour period; and the incorporation of ^{3}H-thymidine into cells of the culture, is measured. The protein has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the protein. Suitable Hodgkin's Disease cell lines to determine the cytostatic or cytotoxic effects of the proteins of the invention are L428, L450, HDLM2 or KM-H2.

Wherein the protein of the invention is an antibody, the antibody is a monoclonal antibody, preferably a recombinant antibody, and most preferably is human, humanized, or chimeric.

The invention further provides isolated nucleic acids encoding a protein that competes for binding to CD30 with monoclonal antibody AC10 or HeFi-1, and exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line. The invention further provides methods of isolating nucleic acids encoding antibodies that immunospecifically bind CD30 and exert a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line.

The invention further provides a method of producing a protein comprising growing a cell containing a recombinant nucleotide sequence encoding a protein, which protein competes for binding to CD30 with monoclonal antibody AC10 or HeFi-1 and exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, such that the protein is expressed by the cell; and recovering the expressed protein.

The invention yet further provides a method for identifying an anti-CD30 antibody useful for the treatment or prevention of Hodgkin's Disease, comprising determining whether the anti-CD30 antibody exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line by contacting a culture of the Hodgkin's Disease cell line with the protein, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours; exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72-hour period; and measuring the incorporation of ^{3}H-thymidine into cells of the culture. The anti-CD30 antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line and is useful for the treatment or prevention of Hodgkin's Disease if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the anti-CD30 antibody.

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. Growth inhibition of Hodgkin's disease cell lines: Hodgkin's disease cell lines HDLM-2, L540, L428 and KM-H2 were cultured at 5×10^4 cells/well in the presence or absence of 10 μg/ml of immobilized AC10. Ki-1 was used as a control in these assays. Proliferation was measured by ^{3}H-thymidine incorporation following 72 hours of culture.

FIG. **2**. Growth inhibition of Hodgkin's disease cell lines: Hodgkin's disease cell lines HDLM-2, L540, L428 and KM-H2 were cultured at 5×10^3 cells/well in the presence or absence of 10 μg/ml of immobilized AC10. Ki-1 was used as a control in these assays. Proliferation was measured by ^{3}H-thymidine incorporation following 72 hours of culture.

FIG. **3**. Growth inhibition of Hodgkin's disease cell lines: Hodgkin's disease cell lines HDLM-2, L540, L428 and KM-H2 were cultured at 5×10^4 cells/well in the presence or absence of 0.1 μg/ml AC10 or HeFi-1 that had been cross-linked by the addition of 20 μg/ml polyclonal goat anti-mouse IgG antibodies. Proliferation was measured by ^{3}H-thymidine incorporation following 72 hours of culture.

FIG. **4**. Growth inhibition of Hodgkin's disease cell lines: Hodgkin's disease cell lines HDLM-2, L540, L428 and KM-H2 were cultured at 5×10^3 cells/well in the presence or absence of 0.11 g/ml AC10 or HeFi-1 that had been cross-linked by the addition of 20 μg/ml polyclonal goat anti-mouse IgG antibodies. Proliferation was measured by ^{3}H-thymidine incorporation following 72 hours of culture.

FIG. **5**. Antitumor activity of AC10 (circles) and HeFi-1 (squares) in disseminated (A) and subcutaneous (B) L540cy Hodgkin's disease xenografts. A) Mice were implanted with 1×10^7 cells through the tail vein on day 0 and received intraperitoneal injections of antibody at 1 mg/kg/injection using an administration schedule of q2dx10. B) Mice were implanted subcutaneously with 2×10^7 L540cy cells. When tumors were palpable mice were treated with intraperitoneal injections of AC10 or HeFi-1 at 2 mg/kg/injection q2dx10. In both experiments untreated mice (X) received no therapy.

FIG. **6**. Antitumor activity of chimeric AC10 (cAC10) in subcutaneous L540cy Hodgkin's disease xenografts. SCID mice were implanted subcutaneously with L540cy cells and when the tumors reached an average size of >150 mm^3 mice were either left untreated (X) or treated with cAC10 (□) at 2 mg/kg twice per week for 5 injections.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to proteins that bind to CD30 and exert a cytostatic or cytotoxic effect on HD cells. The invention further relates to proteins that compete with AC10 or HeFi-1 for binding to CD30 and exert a cytostatic or cytotoxic effect on HD cells. In one embodiment, the protein is an antibody. In a preferred mode of the embodiment, the antibody is AC10 or HeFi-1, most preferably a humanized or chimeric AC10 or HeFi-1.

The invention further relates to proteins encoded by and nucleotide sequences of AC10 and HeFi-1 genes. The invention further relates to fragments and other derivatives and analogs of such AC10 and HeFi-1 proteins. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins, e.g., by recombinant methods, is provided.

The invention also relates to AC10 and HeFi-1 proteins and derivatives including fusion/chimeric proteins which are functionally active, i.e., which are capable of displaying binding to CD30 and exerting a cytostatic or cytotoxic effect on HD cells.

Antibodies to CD30 encompassed by the invention include human, chimeric or humanized antibodies, and such antibodies conjugated to cytotoxic agents such chemotherapeutic drugs.

The invention further relates to methods of treating or preventing HD comprising administering a composition comprising a protein or nucleic acid of the invention alone or in combination with a cytotoxic agent, including but not limited to a chemotherapeutic drug.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1 Proteins of the Invention

The present invention encompasses proteins, including but not limited to antibodies, that bind to CD30 and exert cytostatic and/or cytotoxic effects on HD cells. The invention further relates to proteins that compete with AC10 or HeFi-1 for binding to CD30 and exert a cytostatic or cytotoxic effect on HD cells.

An ATCC deposit has been made on Apr. 26, 2005 pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure. The ATCC is located at 10801 University Boulevard, Manassas, Va. 20110-2209, USA. The ATCC deposit of the hybridoma: mAC10 was given an accession number of PTA-6679. Any deposit is provided as a convenience to those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. Section 112. That described herein is not to be limited in scope by the antibody deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any antibody that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

The present invention further encompasses proteins comprising, or alternatively consisting of, a CDR of HeFi-1 (SEQ ID NO:20, SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32) or AC10 (SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:12; SEQ ID NO:14; or SEQ ID NO:16).

The present invention further encompasses proteins comprising, or alternatively consisting of, a variable region of HeFi-1 (SEQ ID NO:18 or SEQ ID NO:26) or AC10 (SEQ ID NO:2 or SEQ ID NO:10). A table indicating the region of AC10 or HeFi-1 to which each SEQ ID NO corresponds to is provided below:

TABLE 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
AC10 Heavy Chain Variable Region	Nucleotide	1
AC10 Heavy Chain Variable Region	Amino Acid	2
AC10 Heavy Chain-CDR1 (H1)	Nucleotide	3
AC10 Heavy Chain-CDR1 (H1)	Amino Acid	4
AC10 Heavy Chain-CDR2 (H2)	Nucleotide	5
AC10 Heavy Chain-CDR2 (H2)	Amino Acid	6
AC10 Heavy Chain-CDR3 (H3)	Nucleotide	7
AC10 Heavy Chain-CDR3 (H3)	Amino Acid	8
AC10 Light Chain Variable Region	Nucleotide	9
AC10 Light Chain Variable Region	Amino Acid	10
AC10 Light Chain-CDR1 (L1)	Nucleotide	11
AC10 Light Chain-CDR1 (L1)	Amino Acid	12
AC10 Light Chain-CDR2 (L2)	Nucleotide	13
AC10 Light Chain-CDR2 (L2)	Amino Acid	14
AC10 Light Chain-CDR3 (L3)	Nucleotide	15
AC10 Light Chain-CDR3 (L3)	Amino Acid	16
HeFi-1 Heavy Chain Variable Region	Nucleotide	17
HeFi-1 Heavy Chain Variable Region	Amino Acid	18
HeFi-1 Heavy Chain-CDR1 (H1)	Nucleotide	19
HeFi-1 Heavy Chain-CDR1 (H1)	Amino Acid	20
HeFi-1 Heavy Chain-CDR2 (H2)	Nucleotide	21
HeFi-1 Heavy Chain-CDR2 (H2)	Amino Acid	22
HeFi-1 Heavy Chain-CDR3 (H3)	Nucleotide	23
HeFi-1 Heavy Chain-CDR3 (H3)	Amino Acid	24
HeFi-1 Light Chain Variable Region	Nucleotide	25
HeFi-1 Light Chain Variable Region	Amino Acid	26
HeFi-1 Light Chain-CDR1 (L1)	Nucleotide	27
HeFi-1 Light Chain-CDR1 (L1)	Amino Acid	28
HeFi-1 Light Chain-CDR2 (L2)	Nucleotide	29
HeFi-1 Light Chain-CDR2 (L2)	Amino Acid	30
HeFi-1 Light Chain-CDR3 (L3)	Nucleotide	31
HeFi-1 Light Chain-CDR3 (L3)	Amino Acid	32

The present invention further comprises functional derivatives or analogs of AC10 and HeFi-1. As used herein, the term "functional" in the context of a peptide or protein of the invention indicates that the peptide or protein is 1) capable of binding to CD30 and 2) exerts a cytostatic and/or cytotoxic effect on HD cells.

Generally, antibodies of the invention immunospecifically bind CD30 and exert cytostatic and cytotoxic effects on malignant cells in HD. Antibodies of the invention are preferably monoclonal, and may be multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, and CD30 binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds CD30. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

In certain embodiments of the invention, the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and $F(ab')_2$, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, CH3 and CL domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, CH3 and CL domains. Preferably, the antibodies are human,

murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camelid, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries, from human B cells, or from animals transgenic for one or more human immunoglobulin, as described infra and, for example in U.S. Pat. No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multi specificity. Multispecific antibodies may be specific for different epitopes of CD30 or may be specific for both CD30 as well as for a heterologous protein. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., 1991, J. Immunol. 147:60–69; U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., 1992, J. Immunol. 148:1547–1553.

Antibodies of the present invention may be described or specified in terms of the particular CDRs they comprise. In certain embodiments antibodies of the invention comprise one or more CDRs of AC10 and/or HeFi-1. The invention encompasses an antibody or derivative thereof comprising a heavy or light chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs are from monoclonal antibody AC10 or HeFi-1, and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in monoclonal antibody AC 10 or HeFi-1, respectively, and in which said antibody or derivative thereof immunospecifically binds CD30.

In a specific embodiment, the invention encompasses an antibody or derivative thereof comprising a heavy chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:4, 6, or 8 and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in monoclonal antibody AC10, and in which said antibody or derivative thereof immunospecifically binds CD30.

In a specific embodiment, the invention encompasses an antibody or derivative thereof comprising a heavy chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:20, 22 or 24 and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in monoclonal antibody HeFi-1, and in which said antibody or derivative thereof immunospecifically binds CD30.

In a specific embodiment, the invention encompasses an antibody or derivative thereof comprising a light chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:12, 14 or 16, and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in monoclonal antibody AC10, and in which said antibody or derivative thereof immunospecifically binds CD30.

In a specific embodiment, the invention encompasses an antibody or derivative thereof comprising a light chain variable domain, said variable domain comprising (a) a set of three CDRs, in which said set of CDRs comprises SEQ ID NO:28, 30, or 32, and (b) a set of four framework regions, in which said set of framework regions differs from the set of framework regions in monoclonal antibody HeFi-1, and in which said antibody or derivative thereof immunospecifically binds CD30.

Additionally, antibodies of the present invention may also be described or specified in terms of their primary structures. Antibodies having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% and most preferably at least 98% identity (as calculated using methods known in the art and described herein) to the variable regions and AC10 or HeFi-1 are also included in the present invention. Antibodies of the present invention may also be described or specified in terms of their binding affinity to CD30. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10–6 M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8}M, 5×10^{-9}M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, $5\times^{-13}$ M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to CD30 or from exerting a cytostatic or cytotoxic effect on HD cells. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to CD30 can be produced by various procedures well known in the art. For example, CD30 can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the protein. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *corynebacterium parvum*. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed., 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563–681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be

immunized with CD30 or a cell expressing CD30 or a fragment or derivative thereof. Once an immune response is detected, e.g., antibodies specific for CD30 are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding CD30. Ascites fluid, which generally contains high levels of antibodies, can be generated by injecting mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to CD30.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the variable region, the light chain constant region and the CH 1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the nucleic acid sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the nucleic acid sequences encoding them. In particular, DNA sequences encoding V_H and V_L domains are amplified from animal cDNA libraries (e.g., human or murine cDNA libraries of lymphoid tissues). The DNA encoding the V_H and V_L domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector (e.g., p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage expressing an antigen binding domain that binds to CD30 or an AC10 or HeFi-binding portion thereof can be selected or identified with antigen e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., 1995, J. Immunol. Methods 182:41–50; Ames et al., 1995, J. Immunol. Methods 184:177–186; Kettleborough et al., 1994, Eur. J. Immunol. 24:952–958; Persic et al., 1997, Gene 187:9–18; Burton et al., 1994, Advances in Immunology, 191–280; PCT Application No. PCT/GB91/01 134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/1 1236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5;580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 1992, 12(6):864–869; and Sawai et al, 1995, AJRI 34:26–34; and Better et al., 1988, Science 240:1041–1043 (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., 1991, Methods in Enzymology 203:46–88; Shu et al., 1993, PNAS 90:7995–7999; and Skerra et al., 1988, Science 240:1038–1040. For some uses, including in vivo use of antibodies in humans and in vitro proliferation or cytotoxicity assays, it is preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science, 1985, 229:1202; Oi et al., 1986, BioTechniques 4:214; Gillies et al., 1989, J. Immunol. Methods 125:191–202: U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more CDRs from the non-human species and framework and constant regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., 1988, Nature 332:323, which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 9 1/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology, 1991, 28(4/5):489–498; Studnicka et al., 1994, Protein Engineering 7(6):805–814; Roguska. et al., 1994, PNAS 91:969–973), and chain shuffling (U.S. Pat. No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433,

WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of CD30. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see, Lonberg and Huszar, 1995, Int. Rev. Immunol. 13:65–93. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., 1994, Bio/technology 12:899–903).

Further, antibodies to CD30 can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" proteins of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1989, FASEB J. 7(5):437–444; and Nissinoff, 1991, J. Immunol. 147(8): 2429–2438). Fab fragments of such anti-idiotypes can be used in therapeutic regimens to elicit an individual's own immune response against CD30 and HD cells.

As alluded to above, proteins that are therapeutically or prophylactically useful against HD need not be antibodies. Accordingly, proteins of the invention may comprise one or more CDRs from an antibody that binds to CD30 and exerts a cytotoxic and/or cytostatic effect on HD cells. Preferably, a protein of the invention is a multimer, most preferably a dimer.

The invention also provides proteins, including but not limited to antibodies, that competitively inhibit binding of AC10 or HeFi-1 to CD30 as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the protein competitively inhibits binding of AC10 or HeFi-1 to CD30 by at least 50%, more preferably at least 60%, yet more preferably at least 70%, and most preferably at least 75%. In other embodiments, the protein competitively inhibits binding of AC10 or HeFi-1 to CD30 by at least 80%, at least 85%, at least 90%, or at least 95%.

As discussed in more detail below, the proteins of the present invention may be used either alone or in combination with other compositions in the prevention or treatment of HD. The proteins may further be recombinantly fused to a heterologous protein at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to cytotoxic agents, proteins or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as chemotherapeutics or toxins, or comprise a radionuclide for use as a radio-therapeutic. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387.

Proteins of the invention may be produced recombinantly by fusing the coding region of one or more of the CDRs of an antibody of the invention in frame with a sequence coding for a heterologous protein. The heterologous protein may provide one or more of the following characteristics: added therapeutic benefits; promote stable expression of the protein of the invention; provide a means of facilitating high yield recombinant expression of the protein of the invention; or provide a multimerization domain.

In addition to proteins comprising one or more CDRs of an antibody of the invention, proteins of the invention may be identified using any method suitable for screening for protein—protein interactions. Initially, proteins are identified that bind to CD30, then their ability to exert a cytostatic or cytotoxic effect on HD cells can be determined. Among the traditional methods which can be employed are "interaction cloning" techniques which entail probing expression libraries with labeled CD30 in a manner similar to the technique of antibody probing of λgt11 libraries, supra. By way of example and not limitation, this can be achieved as follows: a cDNA clone encoding CD30 (or an AC10 or HeFi-1 binding domain thereof) is modified at the terminus by inserting the phosphorylation site for the heart muscle kinase (HMK) (Blanar & Rutter, 1992, Science 256:1014–1018). The recombinant protein is expressed in *E. coli* and purified on a GDP-affinity column to homogeneity (Edery et al., 1988, Gene 74:517–525) and labeled using γ^{32}P-ATP and bovine heart muscle kinase (Sigma) to a specific activity of 1×10^8 cpm/μg, and used to screen a human placenta λgt11 cDNA library in a "far-Western assay" (Blanar & Rutter, 1992, Science 256:1014–1018). Plaques which interact with the CD30 probe are isolated. The cDNA inserts of positive λ plaques are released and subcloned into a vector suitable for sequencing, such as pBluescript KS (Stratagene).

One method which detects protein interactions in vivo, the two-hybrid system, is described in detail for illustration purposes only and not by way of limitation. One version of this system has been described (Chien et al., 1991, Proc. Natl. Acad. Sci. USA, 88:9578–9582) and is commercially available from Clontech (Palo Alto, Calif.).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to CD30, and the other consists of the activator protein's activation domain fused to an unknown protein that is encoded by a cDNA which has been recombined into this

plasmid as part of a cDNA library. The plasmids are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding sites. Either hybrid protein alone cannot activate transcription of the reporter gene, the DNA-binding domain hybrid cannot because it does not provide activation function, and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodology can be used to screen activation domain libraries for proteins that interact with CD30, which in this context is a "bait" gene product. Total genomic or cDNA sequences are fused to the DNA encoding an activation domain. This library and a plasmid encoding a hybrid of a CD30 coding region (for example, a nucleotide sequence which codes for a domain of CD30 known to interact with HeFi-1 or AC10) fused to the DNA-binding domain are co-transformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, and not by way of limitation, the CD30 coding region can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

Once a CD30-binding protein is identified, its ability (alone or when multimerized or fused to a dimerization or multimerization domain) to elicit a cytostatic or cytotoxic effect on HD cells is determined by contacting a culture of an HD cell line, such as L428, L450, HDLM2 or KM-H2, with the protein. Culture conditions are most preferably about 5,000 cells in a culture area of about 0.33 cm^2, and the contacting period being approximately 72 hours. The culture is then exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of the 72-hour period and the incorporation of ^{3}H-thymidine into cells of the culture is measured. The protein has a cytostatic or cytotoxic effect on the HD cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same cell line cultured under the same conditions but not contacted with the protein.

Without limitation as to mechanism of action, a protein of the invention preferably has more than one CD30-binding site and therefore a capacity to cross link CD30 molecules. Proteins which bind to CD30 or compete for binding to CD30 with AC10 or HeFi-1 can acquire the ability to induce cytostatic or cytotoxic effects on HD cells if dimerized or multimerized. Wherein the CD30-binding protein is a monomeric protein, it can be expressed in tandem, thereby resulting in a protein with multiple CD30 binding sites. The CD30-binding sites can be separated by a flexible linker region. In another embodiment, the CD30-binding proteins can be chemically cross-linked, for example using gluteraldehyde, prior to administration. In a preferred embodiment, the CD30-binding region is fused with a heterologous protein, wherein the heterologous protein comprises a dimerization and multimerization domain. Prior to administration of the protein of the invention to a subject for the purpose of treating or preventing HD, such a protein is subjected to conditions that allows formation of a homodimer or heterodimer. A heterodimer, as used herein, may comprise identical dimerization domains but different CD30-binding regions, identical CD30-binding regions but different dimerization domains, or different CD30-binding regions and dimerization domains.

Particularly preferred dimerization domains are those that originate from transcription factors.

In one embodiment, the dimerization domain is that of a basic region leucine zipper ("bZIP"). bZIP proteins characteristically possess two domains—a leucine zipper structural domain and a basic domain that is rich in basic amino acids, separated by a "fork" domain (C. Vinson et al., 1989, Science, 246:911–916). Two bZIP proteins dimerize by forming a coiled coil region in which the leucine zipper domains dimerize. Accordingly, these coiled coil regions may be used as fusion partners for the proteins and the invention.

Particularly useful leucine zipper domain are those of the yeast transcription factor GCN4, the mammalian transcription factor CCAAT/enhancer-binding protein C/EBP, and the nuclear transform in oncogene products, Fos and Jun (see Landschultz et al., 1988, Science 240:1759–1764; Baxevanis and Vinson, 1993, Curr. Op. Gen. Devel., 3:278–285; and O'Shea et al., 1989, Science, 243:538–542).

In another embodiment, the dimerization domain is that of a basic-region helix-loop-helix ("bHLH") protein (Murre et al, 1989, Cell, 56:777–783). bHLH proteins are also composed of discrete domains, the structure of which allows them to recognize and interact with specific sequences of DNA. The helix-loop-helix region promotes dimerization through its amphipathic helices in a fashion analogous to that of the leucine zipper region of the bZIP proteins (Davis et al., 1990 Cell, 60:733–746; Voronova and Baltimore, 1990 Proc. Natl. Acad. Sci. USA, 87:4722–4726). Particularly useful hHLH proteins are myc, max, and mac.

Heterodimers are known to form between Fos and Jun (Bohmann et al., 1987, Science, 238:1386–1392), among members of the ATF/CREB family (Hai et al., 1989, Genes Dev., 3:2083–2090), among members of the C/EBP family (Cao et al., 1991, Genes Dev., 5:1538–1552; Williams et al., 1991, Genes Dev., 5:1553–1567; and Roman et al., 1990, Genes Dev., 4:1404–1415), and between members of the ATF/CREB and Fos/Jun families Hai and Curran, 1991, Proc. Natl. Acad. Sci. USA, 88:3720–3724). Therefore, when a protein of the invention is administered to a subject as a heterodimer comprising different dimerization domains, any combination of the foregoing may be used.

5.2 Binding Assays

As described above, the proteins, including antibodies, of the invention bind to CD30 and exert a cytostatic or cytotoxic effect on HD cells. Methods of demonstrating the ability of a protein of the invention to bind to CD30 are described herein.

The antibodies of the invention may be assayed for immunospecific binding to CD30 by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as Western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et. al., eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which

is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody to the cell lysate, incubating for a period of time (e.g., 1–4 hours) at 40° C., adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40° C., washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody to immunoprecipitate CD30 can be assessed by, e.g., Western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to CD30 and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al., eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%–20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, incubating the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (i.e., the putative anti-CD30 antibody) diluted in blocking buffer, washing the membrane in washing buffer, incubating the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzyme substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the secondary antibody. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding Western blot protocols see, e.g., Ausubel et al., eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen (i.e., CD30), coating the well of a 96 well microtiter plate with the CD30, adding the antibody conjugated to a detectable compound such as an enzyme (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antibody. In ELISAs the antibody does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of CD30 protein to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al., eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to CD30 and the off-rate of an antibody CD30 interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled CD30 (e.g., ^{3}H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled CD30, and the detection of the antibody bound to the labeled CD30. The affinity of the antibody for CD30 and the binding off-rates can then be determined from the data by Scatchard plot analysis. Competition with a second antibody (such as AC10 or HeFi-1) can also be determined using radioimmunoassays. In this case, CD30 is incubated with the antibody of interest conjugated to a labeled compound (e.g., ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

Proteins of the invention may also be assayed for their ability to bind to CD30 by a standard assay known in the art. Such assays include far Westerns and the yeast two hybrid system. These assays are described in Section 5.2, supra. Another variation on the far Western technique described above entails measuring the ability of a labeled candidate protein to bind to CD30 in a Western blot. In one non-limiting example of a far Western blot, CD30 or the fragment thereof of interest is expressed as a fusion protein further comprising glutathione-S-transferase (GST) and a protein serine/threonine kinase recognition site (such as a cAMP-dependent kinase recognition site). The fusion protein is purified on glutathione-Sepharose beads (Pharmacia Biotech) and labeled with bovine heart kinase (Sigma) and 100 μCi of ^{32}P-ATP (Amersham). The test protein(s) of interest are separated by SDS-PAGE and blotted to a nitrocellulose membrane, then incubated with the labeled CD30. Thereafter, the membrane is washed and the radioactivity quantitated. Conversely, the protein of interest can be labeled by the same method and used to probe a nitrocellulose membrane onto which CD30 has been blotted.

5.3 Assays for Cytotoxic and Cytostatic Activities

By definition, a protein of the invention must exert a cytostatic or cytotoxic effect on a cell of HD. Suitable HD cell lines for this purpose include L428, L450, HDLM2 and KM-H2 (all of which are available from the German Collection of Microorganisms and Cell Cultures (DMSZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH)).

Many methods of determining whether a protein exerts a cytostatic or cytotoxic effect on a cell are known to those of skill in the art, and can be used to elucidate whether a particular protein is a protein of the invention. Illustrative examples of such methods are described below.

Wherein a protein that binds to CD30 does not exert a cytostatic or cytotoxic effect on HD cells, the protein can be multimerized according to the methods described in Section 5.1, supra, and the multimer assayed for its ability to exert a cytostatic or cytotoxic effect on HD cells.

Once a protein is identified that both (i) binds to CD30 and (ii) exerts a cytostatic or cytotoxic effect on HD cells, its therapeutic value is validated in an animal model, as described in Section 6, infra.

In a preferred embodiment, determining whether a protein exerts a cytostatic or cytotoxic effect on a HD cell line can be made by contacting a 5,000 cell-culture of the HD cell line in a culture area of about 0.33 cm^2 with the protein for

a period of 72 hours. During the last 8 hours of the 72-hour period, the culture is exposed to 0.5 μCi of ^{3}H-thymidine. The incorporation of ^{3}H-thymidine into cells of the culture is then measured. The protein has a cytostatic or cytotoxic effect on the HD cell line and is useful for the treatment or prevention of HD if the cells of the culture contacted with the protein have reduced ^{3}H-thymidine incorporation compared to cells of the same HD cell line cultured under the same conditions but not contacted with the anti-CD30 antibody.

There are many cytotoxicity assays known to those of skill in the art. Some of these assays measure necrosis, while others measure apoptosis (programmed cell death). Necrosis is accompanied by increased permeability of the plasma membrane; the cells swell and the plasma membrane ruptures within minutes. On the other hand, apoptosis is characterized by membrane blebbing, condensation of cytoplasm and the activation of endogenous endonucleases. Only one of these effects on HD cells is sufficient to show that a CD30-binding protein is useful in the treatment or prevention of HD as an alternative to the assays measuring cytostatic or cytotoxic effects described above.

In one embodiment, necrosis measured by the ability or inability of a cell to take up a dye such as neutral red, trypan blue, or ALAMAR™ blue (Page et al., 1993, Intl. J. of Oncology 3:473–476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically.

In another embodiment, the dye is sulforhodamine B (SRB), whose binding to proteins can be used as a measure of cytotoxicity (Skehan et al., 1990, J. Nat'l Cancer Inst. 82:1107–12).

In yet another embodiment, a tetrazolium salt, such as MTT, is used in a quantitative colorimetric assay for mammalian cell survival and proliferation by detecting living, but not dead, cells (see, e.g., Mosmann, 1983, J. Immunol. Methods 65:55–63).

In yet another embodiment, apoptotic cells are measured in both the attached and "floating" compartments of the cultures. Both compartments are collected by removing the supernatant, trypsinizing the attached cells, and combining both preparations following a centrifugation wash step (10 minutes, 2000 rpm). The protocol for treating tumor cell cultures with sulindac and related compounds to obtain a significant amount of apoptosis has been described in the literature (see, e.g., Piazza et al., 1995, Cancer Research 55:3110–16). Features of this method include collecting both floating and attached cells, identification of the optimal treatment times and dose range for observing apoptosis, and identification of optimal cell culture conditions.

In yet another embodiment, apoptosis is quantitated by measuring DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica, 1999, no. 2, pp. 34–37 (Roche Molecular Biochemicals).

In yet another embodiment, apoptosis can be observed morphologically.

Following treatment with a test protein or nucleic acid, cultures can be assayed for apoptosis and necrosis by fluorescent microscopy following labeling with acridine orange and ethidium bromide. The method for measuring apoptotic cell number has previously been described by Duke & Cohen, 1992, Current Protocols In Immunology, Coligan et al., eds., 3.17.1–3.17.16. In another mode of the embodiment, cells can be labeled with the DNA dye propidium iodide, and the cells observed for morphological changes such as chromatin condensation and margination along the inner nuclear membrane, cytoplasmic condensation, increased membrane blebbing and cellular shrinkage.

5.4 Nucleic Acids of the Invention

The invention further provides nucleic acids comprising a nucleotide sequence encoding a protein, including but not limited to, a protein of the invention and fragments thereof. Nucleic acids of the invention preferably encode one or more CDRs of antibodies that bind to CD30 and exert cytotoxic or cytostatic effects on HD cells. Exemplary nucleic acids of the invention comprise SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:29 or SEQ ID NO:31. Preferred nucleic acids of the invention comprise SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:17, or SEQ ID NO:25. (See Table 1 at pages 9–10, supra, for identification of the domain of AC10 or HeFi-1 to which these sequence identifiers correspond).

The invention also encompasses nucleic acids that hybridize under stringent, moderate or low stringency hybridization conditions, to nucleic acids of the invention, preferably, nucleic acids encoding an antibody of the invention.

By way of example and not limitation, procedures using such conditions of low stringency for regions of hybridization of over 90 nucleotides are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. U.S.A. 78,:6789–6792). Filters containing DNA are pretreated for 6 hours at 40° C. in a solution containing 35% formamide, 5×SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5–20×10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18–20 h at 40° C., and then washed for 1.5 h at 55° C. in a solution containing 2×SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60° C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65–68° C. and re-exposed to film. Other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations).

Also, by way of example and not limitation, procedures using such conditions of high stringency for regions of hybridization of over 90 nucleotides are as follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65° C. in buffer composed of 6×SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65° C. in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5–20×10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37° C. for 1 h in a solution containing 2×SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1×SSC at 50° C. for 45 min before autoradiography.

Other conditions of high stringency which may be used depend on the nature of the nucleic acid (e.g. length, GC content, etc.) and the purpose of the hybridization (detec-

tion, amplification, etc.) and are well known in the art. For example, stringent hybridization of a nucleic acid of approximately 15–40 bases to a complementary sequence in the polymerase chain reaction (PCR) is done under the following conditions: a salt concentration of 50 mM KCl, a buffer concentration of 10 mM Tris-HCl, a Mg^{2+} concentration of 1.5 mM, a pH of 7–7.5 and an annealing temperature of 55–60° C.

In another specific embodiment, a nucleic acid which is hybridizable to a nucleic acid of the invention acid, or its complement, under conditions of moderate stringency is provided. Selection of appropriate conditions for such stringencies is well known in the art (see e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; see also, Ausubel et al., eds., in the Current Protocols in Molecular Biology series of laboratory technique manuals, © 1987–1997, Current Protocols, © 1994–1997 John Wiley and Sons, Inc.).

The nucleic acids of the invention may be obtained, and the nucleotide sequence of the nucleic acids determined, by any method known in the art. For example, if the nucleotide sequence of the protein is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the protein, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a nucleic acid encoding a protein of the invention may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular protein is not available, but the sequence of the protein molecule is known, a nucleic acid encoding the protein may be chemically synthesized or obtained from a suitable source (e.g., a cDNA library such as an antibody cDNA library or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the protein. If the protein is an antibody, the library source can be hybridoma cells selected to express the antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the protein. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the protein may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the protein is an antibody, and the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the CDRs by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and are preferably human framework regions (see, e.g., Chothia et al., 1998, J. Mol. Biol. 278:457–479 for a listing of human framework regions). The nucleic acid generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds CD30 and exerts a cytostatic and/or cytotoxic effect on HD cells. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to CD30 and/or to enhance the cytostatic and/or cytotoxic effect of the antibody. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the nucleic acid are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851–855; Neuberger et al., 1984, Nature 312:604–608; Takeda et al., 1985, Nature 314:452–454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423–42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879–5883; and Ward et al., 1989, Nature 334:544–54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain protein. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., 1988, Science 242:1038–1041).

5.5 Sequences Related to AC10 and HeFi-1

The present invention further encompasses proteins and nucleic acids comprising a region of homology to CDRs of AC10 and HeFi-1, or the coding regions therefor, respectively. In various embodiments, the region of homology is characterized by at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% identity with the corresponding region of AC10 or HeFi-1.

In one embodiment, the present invention provides a protein with a region of homology to a CDR of HeFi-1 (SEQ ID NO:20, SEQ ID NO:22; SEQ ID NO:24; SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32). In another embodiment, the present invention provides a protein with a region of homology to a CDR of AC10 (SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:12; SEQ ID NO:14; or SEQ ID NO:16).

In another embodiment, the present invention provides a nucleic acid with a region of homology to a CDR coding region of HeFi-1 (SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:29 or SEQ ID NO:31). In yet another embodiment, the present invention provides a nucleic acid with a region of homology to a CDR coding region of AC10 (SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15).

The present invention further encompasses proteins and nucleic acids comprising a region of homology to the variable regions of AC10 and HeFi-1, or the coding region therefor, respectively. In various embodiments, the region of homology is characterized by at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% identity with the corresponding region of AC10 or HeFi-1.

In one embodiment, the present invention provides a protein with a region of homology to a variable region of HeFi-1 (SEQ ID NO:18 or SEQ ID NO:26). In another embodiment, the present invention provides a protein with a region of homology to a variable region of AC10 (SEQ ID NO: 2 or SEQ ID NO: 10).

In one embodiment, the present invention provides a nucleic acid with a region of homology to a variable region coding region of HeFi-1(SEQ ID NO:17 or SEQ ID NO:25). In another embodiment, the present invention provides a nucleic with a region of homology to a variable region coding region of AC10 (SEQ ID NO:1 or SEQ ID NO:9).

To determine the percent identity of two amino acid sequences or of two nucleic acids, e.g. between the sequences of an AC10 or HeFi-1 variable region and sequences from other proteins with regions of homology to the AC10 or HeFi-1 variable region, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity=# of identical positions/total # of positions (e.g., overlapping positions)×100). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 90:5873–5877. Such an algorithm is incorporated into the NBLAST and the XBLAST programs of Altschul, et al., 1990, J. Mol. Biol. 215:403–410. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid encoding a SCA-1 modifier protein. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to SCA-1 modifier protein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389–3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, 1994, Comput. Appl. Biosci., 10:3–5; and FASTA described in Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. 85:2444–8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. Ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA.

Alternatively, protein sequence alignment may be carried out using the CLUSTAL W algorithm, as described by Higgins et al., 1996, Methods Enzymol. 266:383–402.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

5.6 Methods of Producing the Proteins of the Invention

The proteins, including antibodies, of the invention can be produced by any method known in the art for the synthesis of proteins, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of a protein of the invention, including a fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention) requires construction of an expression vector containing a nucleic acid that encodes the protein. Once a nucleic acid encoding a protein of the invention has been obtained, the vector for the production of the protein molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a nucleic acid containing nucleotide sequence encoding said protein are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding a protein of the invention operably linked to a promoter. Wherein the protein is an antibody, the nucleotide sequence may encode a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then

cultured by conventional techniques to produce a protein of the invention. Thus, the invention encompasses host cells containing a nucleic acid encoding a protein of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the proteins molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express a protein of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces, Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecules, are used for the expression of a recombinant protein of the invention. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for proteins of the invention (Foecking et al., 1986, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the folding and post-translation modification requirements protein being expressed. Where possible, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising a protein of the invention, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO 1. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101–3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503–5509); and the like. pGEX vectors may also be used to express fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of the protein of the invention may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the protein of the invention in infected hosts. (See, e.g., Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 8 1:355–359). Specific initiation signals may also be required for efficient translation of inserted coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:51–544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein of the invention. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, Hela, COS, MDCK, 293, 3T3, and W138.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the protein of the invention may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1–2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the protein of the invention.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22:8–17) genes can be employed in tk-, hgprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, Proc. Natl. Acad. Sci. USA 77:357; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Clinical Pharmacy 12:488–505; Wu and Wu, 1991, Biotherapy 3:87–95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573–596; Mulligan, 1993, Science 260:926–932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62: 191–217; May, 1993, TIB TECH 11(5):155–215); and hygro, which confers resistance to hygromycin (Santerre et al., 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, J. Mol. Biol. 150:1, which are incorporated by reference herein in their entireties.

The expression levels of a protein of the invention can be increased by vector amplification (for a review, see Bebbington and Hentschel, "The Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNY Cloning", Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the protein of the invention will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

Wherein the protein of the invention is an antibody, the host cell may be co-transfection with two expression vectors of the invention, the first vector encoding a heavy chain derived protein and the second vector encoding a light chain derived protein. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain proteins. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain proteins. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52 (1986); Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2 197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once a protein molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of proteins, for example, by chromatography (e.g., ion exchange; affinity, particularly by affinity for the specific antigen, Protein A (for antibody molecules, or affinity for a heterologous fusion partner wherein the protein is a fusion protein; and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

The present invention encompasses CD3-binding proteins recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugation) to heterologous proteins (of preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or at least 100 amino acids) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences.

The present invention further includes compositions comprising proteins of the invention fused or conjugated to antibody domains other than the variable regions. For example, the proteins of the invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a protein of the invention may comprise the constant region, hinge region, CH 1 domain, CR2 domain, and CH3 domain or any combination of whole domains or portions thereof. The proteins may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the proteins of the invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the proteins to portions of IgA and IgM. Methods for fusing or conjugating the proteins of the invention to antibody portions are known in the art. See, e.g., U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 9 1/06570; Ashkenazi et al., 1991, Proc. Nat. Acad. Sci. USA 88:10535–10539; Zheng et al., 1995, J. Immunol. 154:5590–5600; and Vil et al., 1992, Proc. Natl. Acad. Sci. USA 89:11337–11341 (said references incorporated by reference in their entireties).

5.7 Conjugates and Fusion Proteins

As discussed, supra, the proteins of the invention encompass proteins that bind to CD30 and exert a cytostatic and/or cytotoxic effect on HD cells, and that are further fused or conjugated to heterologous proteins or cytotoxic agents.

The present invention thus provides for treatment of Hodgkin's Disease by administration of a protein or nucleic acid of the invention. Proteins of the invention include but are not limited to: AC10 and HeFi-1 proteins, antibodies and analogs and derivatives thereof (e.g., as described herein above); the nucleic acids of the invention include but are not limited to nucleic acids encoding such AC10 and HeFi-1 proteins, antibodies and analogs or derivatives (e.g., as described herein above).

In certain embodiments of the invention, a protein or nucleic acid of the invention may be chemically modified to improve its cytotoxic and/or cytostatic properties. For example, a protein of the invention can be administered as a conjugate. Particularly suitable moieties for conjugation to proteins of the invention are chemotherapeutic agents, prodrug converting enzymes, radioactive isotopes or compounds, or toxins. Alternatively, a nucleic acid of the invention may be modified to functionally couple the coding sequence of a pro-drug converting enzyme with the coding sequence of a protein of the invention, such that a fusion protein comprising the functionally active pro-drug converting enzyme and protein of the invention is expressed in the subject upon administration of the nucleic acid in accordance with the gene therapy methods described in Section 5.7, infra.

In one embodiment, a protein of the invention is fused to a marker sequence, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., 1989, Proc. Natl. Acad. Sci. USA 86:821–824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767) and the "flag" tag. Such fusion proteins can be generated by standard recombinant methods known to those of skill in the art.

In another embodiment, the proteins of the invention are fused or conjugated to a therapeutic agent. For example, a protein of the invention may be conjugated to a cytotoxic agent such as a chemotherapeutic agent, a toxin (e.g., a cytostatic or cytocidal agent), or a radionuclide (e.g., alpha-emitters such as, for example, ^{212}Bi, ^{211}At, or beta-emitters such as, for example, ^{131}I, ^{90}Y, or ^{67}Cu).

Drugs such as methotrexate (Endo et al., 1987, Cancer Research 47:1076–1080), daunomycin (Gallego et al., 1984, Int. J. Cancer. 33:737–744), mitomycin C (MMC) (Ohkawa et al., 1986, Cancer Immunol. Immunother. 23:81–86) and vinca alkaloids (Rowland et al., 1986, Cancer Immunol Immunother. 21:183–187) have been attached to antibodies and the derived conjugates have been investigated for antitumor activities. Care should be taken in the generation of chemotherapeutic agent conjugates to ensure that the activity of the drug and/or protein does not diminish as a result of the conjugation process.

Examples of chemotherapeutic agents include the following non-mutually exclusive classes of chemotherapeutic agents: alkylating agents, anthracyclines, antibiotics, antifolates, antimetabolites, antitubulin agents, auristatins, chemotherapy sensitizers, DNA minor groove binders, DNA replication inhibitors, duocarmycins, etoposides, fluorinated pyrimidines, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, and vinca alkaloids. Examples of individual chemotherapeutics that can be conjugated to a nucleic acid or protein of the invention include but are not limited to an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluordeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbizine, streptozotocin, tenoposide, 6-thioguanine, thio TEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

The conjugates of the invention used for enhancing the therapeutic effect of the protein of the invention include non-classical therapeutic agents such as toxins. Such toxins include, for example, abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

Techniques for conjugating such therapeutic moieties to proteins, and in particular to antibodies, are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243–56 (Alan R. Liss, Inc., 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd ed.), Robinson et al. (eds.), pp. 623–53 (Marcel Dekker, Inc., 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303–16 (Academic Press 1985), and Thorpe et al., 1982, Immunol. Rev. 62:119–58.

Alternatively, an antibody of the invention can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, which is incorporated herein by reference in its entirety.

As discussed above, in certain embodiments of the invention, a protein of the invention can be co-administered with a pro-drug converting enzyme. The pro-drug converting enzyme can be expressed as a fusion protein with or conjugated to a protein of the invention. Exemplary pro-drug converting enzymes are carboxypeptidase G2, beta-glucuronidase, penicillin-V-amidase, penicillin-G-amidase, β-lactamase, β-glucosidase, nitroreductase and carboxypeptidase A.

5.8 Gene Therapy

In a specific embodiment, nucleic acids of the invention are administered to treat, inhibit or prevent HD. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see, Goldspiel et al., 1993, Clinical Pharmacy 12:488–505; Wu and Wu, 1991, Biotherapy 3:87–95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573–596; Mulligan, 1993, Science 260:926–932; Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191–217; May, 1993, TIBTECH 1, 1(5):155–215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the therapeutic comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932–8935; Zijlstra et al., 1989, Nature 342: 435–438. In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic

acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, for example by constructing them as part of an appropriate nucleic acid expression vector and administering the vector so that the nucleic acid sequences become intracellular. Gene therapy vectors can be administered by infection using defective or attenuated retrovirals or other viral vectors (see, e.g., U.S. Pat. No. 4,980,286); direct injection of naked DNA; use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont); coating with lipids or cell-surface receptors or transfecting agents; encapsulation in liposomes, microparticles, or microcapsules; administration in linkage to a peptide which is known to enter the nucleus; administration in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262: 4429–4432) (which can be used to target cell types specifically expressing the receptors); etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06 180; WO 92/22635; W092/20316; W093/14188, and WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932–8935; Zijlstra et al., 1989, Nature 342:435–438).

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581–599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, thereby facilitating delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:29 1–302, which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest. 93:644–651; Klein et al., 1994, Blood 83:1467–1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129–141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110–114.

Another approach to gene therapy involves transferring a gene, e.g. an AC10 or HeFi-1 gene, to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217:599–618; Cohen et al., 1993, Meth. Enzymol. 217:618–644; Cline, 1985, Pharmac. Ther. 29:69–92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to fibroblasts; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973–985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of an protein or pharmaceutical composition include determining the effect of the protein or pharmaceutical composition on a Hodgkin's cell line or a tissue sample from a patient with Hodgkin's Disease. The cytotoxic and/or cytostatic effect of the protein or composition on the Hodgkin's cell line and/or tissue

sample can be determined utilizing techniques known to those of skill in the art. A preferred method, described in Section 6 infra, entails contacting a culture of the Hodgkin's Disease cell line grown at a density of approximately of about 5,000 cells in a 0.33 cm^2 of culture area for a period of 72 hours with the protein or pharmaceutical composition, exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72-hour period, and measuring the incorporation of ^{3}H-thymidine into cells of the culture. The protein or pharmaceutical composition has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line and is useful for the treatment or prevention of Hodgkin's Disease if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the protein or pharmaceutical composition. Alternatively, in vitro assays which can be used to determine whether administration of a specific protein or pharmaceutical composition is indicated, include in vitro cell culture assays in which a tissue sample from a Hodgkin's Disease patient is grown in culture, and exposed to or otherwise a protein or pharmaceutical composition, and the effect of such compound upon the Hodgkin's tissue sample is observed.

5.9 Therapeutic/Prophylactic Administration and Compositions

The invention provides methods of treatment and prophylaxis by administration to a subject of an effective amount of a CD30-binding protein which has a cytotoxic or cytostatic effect on Hodgkin's Disease cells (i.e., a protein of the invention), a nucleic acid encoding said CD30-binding protein (i.e., a nucleic acid of the invention), or a pharmaceutical composition comprising a protein or nucleic acid of the invention (hereinafter, a pharmaceutical of the invention). According to the present invention, treatment of HD encompasses the treatment of patients already diagnosed as HD at any clinical stage; such treatment resulting in delaying tumor growth; and/or promoting tumor regression.

In a preferred embodiment, the protein of the invention is the monoclonal antibody AC10 or HeFi-1 or a fragment or derivative thereof. In a preferred aspect, a pharmaceutical of the invention comprises a substantially purified protein or nucleic acid of the invention (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a nucleic acid or protein of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429–4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. Nucleic acids and proteins of the invention may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents such as chemotherapeutic agents (see Section). Administration can be systemic or local.

In a specific embodiment, it may be desirable to administer the nucleic acid or protein of the invention by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527–1533; Treat et al., 1989, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353–365; Lopez-Berestein, ibid., pp. 317–327; see generally, ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1989, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, 1974, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla.; Controlled Drug Bioavailability, Drug Product Design and Performance, 1984, Smolen and Ball (eds.), Wiley, New York; Ranger and Peppas, 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105).

Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527–1533.

In a specific embodiment where a nucleic acid of the invention is administered, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864–1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

As alluded to above, the present invention also provides pharmaceutical compositions (pharmaceuticals of the invention). Such compostions comprise a therapeutically effective amount of a nucleic acid or protein of the invention, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical com-

position is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the nucleic acid or protein of the invention, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the pharmaceutical of the invention is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical of the invention may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical of the invention is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical of the invention is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The amount of the nucleic acid or protein of the invention which will be effective in the treatment or prevention of HD can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the stage of HD, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

5.10 Kits

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a nucleic acid or protein of the invention and optionally one or more pharmaceutical carriers. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

In one embodiment, a kit comprises a purified protein of the invention. In a preferred mode of the embodiment, the protein is an antibody. The protein may be conjugated to a radionuclide or chemotherapeutic agent. The kit optionally further comprises a pharmaceutical carrier.

In another embodiment, a kit of the invention comprises a nucleic acid of the invention, or a host cell comprising a nucleic acid of the invention, operably linked to a promoter for recombinant expression.

5.11 Effective Dose

Toxicity and therapeutic efficacy of the proteins of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Proteins that exhibit large therapeutic indices are preferred. While proteins that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such proteins to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such proteins lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Generally, the dosage of a protein of the invention in a pharmaceutical of the invention administered to a Hodgkin's Disease patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign proteins. Thus, lower dosages of humanized, chimeric or human antibodies and less frequent administration is often possible.

5.12 Formulations

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the proteins and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate) lubricants (e.g.,

magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicles before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the proteins for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The proteins may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The proteins may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the proteins may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the proteins may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration preferably for administration to a human.

5.13 Combination Therapy for Treatment of Hodgkin's Disease

The nucleic acids and proteins of the invention can be administered together with treatment with irradiation or one or more chemotherapeutic agents.

For irradiation treatment, the irradiation can be gamma rays or X-rays. For a general overview of radiation therapy, see Hellman, Chapter 12: Principles of Radiation Therapy Cancer, in: Principles and Practice of Oncology, DeVita et al., eds., 2nd. Ed., J.B. Lippencott Company, Philadelphia.

Useful classes of chemotherapeutic agents include, but are not limited to, the following non-mutually exclusive classes of agents: alkylating agents, anthracyclines, antibiotics, antifolates, antimetabolites, antitubulin agents, auristatins, chemotherapy sensitizers, DNA minor groove binders, DNA replication inhibitors, duocarmycins, etoposides, fluorinated pyrimidines, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, and vinca alkaloids. Individual chemotherapeutics encompassed by the invention include but are not limited to an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluordeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbizine, streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

In a specific embodiment, a nucleic acid or protein of the invention is administered concurrently with radiation therapy or one or more chemotherapeutic agents.

In another specific embodiment, chemotherapy or radiation therapy is administered prior or subsequent to administration of a nucleic acid or protein of the invention, by at least an hour and up to several months, for example at least an hour, five hours, 12 hours, a day, a week, a month, or three months, prior or subsequent to administration of a nucleic acid or protein of the invention.

In a specific embodiment in which a protein of the invention is conjugated to a pro-drug converting enzyme, or in which a nucleic acid of the invention encodes a fusion protein comprising a pro-drug converting enzyme, the protein or nucleic acid is administered with a pro-drug. Administration of the pro-drug can be concurrent with administration of the nucleic acid or protein of the invention, or, more preferably, follows the administration of the nucleic acid or protein of the invention by at least an hour to up to one week, for example about five hours, 12 hours, or a day. Depending on the pro-drug converting enzyme administered, the pro-drug can be a benzoic acid mustard, an aniline mustard, a phenol mustard, p-hydroxyaniline mustard-glucuronide, epirubicin-glucuronide, adriamycin-N phenoxyaceryl, N-(4'-hydroxyphenyl acetyl)-palytoxin doxorubicin, melphalan, nitrogen mustard-cephalosporin, α-phenylenediamine, vinblastine derivative-cephalosporin, cephalosporin mustard, cyanophenylmethyl-⊕-D-gluco-pyranosiduronic acid, 5-(adaridin-1-yl-)2,4-dinitrobenzamide, or methotrexate-alanine.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from

the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

The invention is further described in the following examples which are in no way intended to limit the scope of the invention.

6. EXAMPLE:

Anti-CD30 Monoclonal Antibodies AC10 and HeFi-1 Inhibit the Growth of CD30 Expressing Hodgkin's Disease Cell Lines

6.1 Materials and Methods

Cells and culture conditions: The CD30 expressing cell lines, L540, HDLM2, L428, KM-H2 and Karpas 299. were obtained from the German Collection of Microorganisms and Cell Cultures/DSMZ in Braunschweig, Germany. The Hodgkin's cell line L540cy was a provided by Dr. V. Diehl of the University of Cologne, Cologne, Germany. The cell lines were maintained in the recommended media formulations and subcultured every 3–4 days.

Reagents and antibodies: Anti-CD30 monoclonal antibody hybridoma line AC10 was described by Bowen et al. (Bowen et al., 1993, J. Immunol. 151:5896–5906). Purified antibody was isolated from serum-free supernatants using a protein-G immunoaffinity column. The resulting AC10 antibody was determined to be >97% monomeric by size exclusion chromatography. The monoclonal antibody HeFi-1 has been previously described and was provided by Dr. T. Hecht, NCI, Bethesda, Md. HeFi-1 mAb was demonstrated by size exclusion chromatography to be greater than 98% monomer.

Proliferation assays: CD30 expressing cell lines were cultured in flat-bottom 96-well plates at a density of 50,000 or 5,000 cells/well in growth media (RPMI with 10% fetal bovine serum (FBS) for cell lines L428, KM-H2 and Karpas 299, and RPMI/20% FBS for cell lines HDLM-2 and L540. The cell lines were cultured in the absence or presence of cross-linked soluble anti-CD30 mAbs or immobilized anti-CD30 mAbs, as described below.

Antibody cross-linking in solution: To cross-link the anti-CD30 antibodies in solution, various dilutions of AC10 or HeFi-1 were titrated into 96-well flat bottom tissue culture plates in the absence or presence of 20 μg/ml polyclonal goat anti-mouse IgG antibodies. Hodgkin's disease cell lines were then added to the plates at either 50,000 or 5,000 cells/well. The plates were incubated at 37° C. for 72 hours and were labeled with ^{3}H-thymidine, 1 μCi/well, for the final 5 hours.

Antibody immobilization: Antibody immobilization was obtained by coating wells with antibody in 50 mmol/L Tris buffer (pH 8.5) for 18 hours at 4° C. Prior to the addition of cells, wells were washed twice with PBS to remove unbound mAb. 50,000 or 5,000 cells in a total volume of 200 μl were added to each well. Proliferation was determined by uptake of ^{3}H-thymidine (0.5 μCi/well) during the final 8 hours of a 72 hour culture period.

6.2 Results

To evaluate the biologic activity of anti-CD30 mAbs, CD30-expressing HD cell lines (50,000 cells/well) were cultured in the presence of immobilized anti-CD30 mAb AC10. mAb AC10 demonstrated inhibition of cell growth of T-cell-like (L540 and HDLM-2) or B-cell-like (L428 and KM-H2) HD lines (FIG. 1). Ki-1, which was previously shown to have no effect on HD cell lines (Gruss et al., 1996, Blood 83:2045–2056), was used as a control.

To further evaluate the activity of AC10, a second series of assays were performed. In order to assess the activity of the AC10 during a period of logarithmic tumor cell growth, the cell density of the cultures was decreased to provide more optimal growth conditions. To that end, HD cell lines were cultured in flat-bottom 96 well plates at a density of 5,000 cells/well in the presence or absence of mAb AC10. AC10 demonstrated growth inhibition of all four HD cell lines tested (L540, HDLM-2, L428 and KM-H2; FIG. 2).

In another set of experiments, HD cell lines were incubated with soluble AC10 or HeFi-1 that were cross-linked in solution by the addition of soluble goat anti-mouse IgG antibodies. Under these cross-linking conditions, all four HD cell lines, when plated at 5×10^4 cell/well, were growth inhibited by AC10 and HeFi-1 (FIG. 3). When the cells were plated at 5×10^3 cell/well, all four HD cell lines were growth inhibited by AC10, while three of the four cell lines, HDLM-2, L540, and L428, were growth inhibited by HeFi-1 (FIG. 4).

The data resulting from the experiments testing the effects of AC10 and HeFi-1 on CD30-expressing tumor cell lines are summarized in Table 2, infra. Table 2 further provides a comparison of the anti-tumor activity of AC10 and HeFi-1 with that of mAb M44.

TABLE 2

Cytostatic and/or cytotoxic activity of signaling anti-CD30 mAbs on CD30-expressing malignant cell lines

		Inhibition of Growth by		
Cell Line	Cell Type	M44[a]	HeFi-1	AC10
Karpas 299	ALCL	+	+	+
Michel	ALCL	+	ND	ND
KM-H2	HD (B cell phenotype)	-	+	+
L428	HD (B cell phenotype)	-	+	+
HDLM-2	HD (T cell phenotype)	-	+	+
L540	HD (T cell phenotype)	-	+	+

[a]Published data from Gruss et al, Blood 83(8): 2045–2056

Taken together, these data indicate that mAbs AC10 and HeFi-1 are distinguished from the previously described anti-CD30 mAbs by their ability to inhibit the growth of CD30-expressing HD lines. It is of interest to note that Hubinger et al. recently evaluated the activity of the anti-CD30 mAb M44, in immobilized form, in a proliferation assay utilizing 5,000 cells/well. Under these conditions, M44 inhibited the growth of the CD30-expressing ALCL line, Karpas 299 but not the HD cell line HDLM-2 (Hubinger et al., 1999, Exp. Hematol. 27(12):1796–805).

7. AC10 Enhances the Cytotoxic Effect of Chemotherapeutics on Hodgkin's Disease Cell Lines

7.1 Materials and Methods

L428 cells were cultured for 24 hours in the presence or absence of 0.1 μg/ml anti-CD30 antibody, AC10, crosslinked by the addition of 20 μg/ml goat anti-mouse IgG antibodies. After the 24-hour culture period, the cells were harvested and washed with phosphate buffered saline (PBS). The cells were then plated into 96-well flat-bottom tissue culture plates at 5×10^3 cells/well and mixed with various dilutions of chemotherapeutic drugs. After a 1-hour exposure to the drugs the cells were washed twice, followed by the addition of fresh culture media. The plates were then incubated at 37° C. for 72 hours followed by a 4-hour incubation with 0.5 μCi/well ^{3}H-thymidine. The inhibition

of growth was determined by comparing the amount of ^{3}H-thymidine incorporated into treated cells to the amount incorporated into untreated control cells.

7.2 Results

To evaluate the effect of the anti-CD30 mAb in combination with chemotherapeutic drugs, L428 cells were incubated for 24 hours in either the absence of antibody or the presence of AC10 at 0.1 μg/ml with 20 μg/ml goat anti-mouse IgG to provide crosslinking for the primary antibody. After this incubation the cells were plated into 96-well tissue culture plates at 5×10^3 cells/well in the presence of dilutions of chemotherapeutic drugs including doxorubicin, cisplatin, and etoposide (Table 3). The EC_{50}, concentration of drug needed to inhibit the incorporation of ^{3}H-thymidine by 50% compared to untreated control cells, was then determined for cells treated with the drugs alone or the combinations of drug and antibody. For doxorubicin, incubation with AC 10 decreased the EC_{50} on L428 cells (i.e. decreased the amount of drug necessary to inhibit 50% of DNA synthesis) from approximately 45 nM (doxorubicin alone) to approximately 9 nM, for cisplatin AC10 decreased the EC_{50} from ~1,500 nM to ~500 nM, and for etoposide AC10 decreased the EC_{50} from ~1,500 nM to ~600 nM.

TABLE 3

AC10 enhances the effectiveness of chemotherapeutic drugs on the HD cell line L428.

	EC_{50}, nM	
Drug	with AC10	without AC10
Doxorubicin	45	9
Cisplatin	1,500	500
Etoposide	1,500	600

8. Antitumor Activity of AC10 and HeFi-1 in Disseminated and Localized (Subcutaneous) L540CY Hodgkin's Disease Xenografts

8.1 Materials and Methods

Human tumor xenograft models: Female C.B-17 SCID mice, obtained from Taconic (Germantown, N.Y.) at 4–6 weeks of age, were used for all efficacy studies. To establish xenograft models of Hodgkin's disease, L540cy (HD) cells were harvested from cell culture, washed in ice cold phosphate buffered saline (PBS), resuspended in PBS, and maintained on ice until implantation. For disseminated disease models, mice were injected intravenously through the tail vein with 10^7 L540cy cells. Solid tumor xenografts were established by injecting mice subcutaneously (s.c.) with 2×10^7 L540cy cells. For therapeutic evaluation the indicated treatment doses and schedules were used.

Administration of AC10 and HeFi-1: Disseminated L540cy tumor bearing mice received 10^7 cells through the tail vein on d0 followed by therapy initiated on d1. Treated mice received i.p. injections of either AC10 or HeFi-1 every two days for a total of 10 injections, q2dx10, at 1 mg/kg/injection.

For the subcutaneous L540cy model, mice were injected s.c. with 2×10^7 cells and were observed daily for solid tumor formation. When tumors were palpable, the animals were randomly distributed into groups and received either AC10 or HeFi-1 q2dx10 at 2 mg/kg/injection.

8.2 Results

AC10 and HeFi-1 were tested in L540cy Hodgkin's disease xenografted SCID mice, as described above. In the mouse population with disseminated L540cy tumors, all of the untreated control animals developed signs of severe disseminated disease such as hind limb paralysis or the formation of a solid tumor mass and had to be sacrificed (mean survival time=37 days). In contrast, all of the mice that received either AC10 or HeFi-1 survived for >46 days with no signs of disease (FIG. 5A).

With respect to the mouse population with subcutaneous L540cy tumors, while the untreated control tumors rapidly grew to >450 mm^3, both mAbs significantly delayed tumor growth as shown in FIG. 5B.

The inventors have identified murine monoclonal antibodies (mAbs) which target the human CD30 receptor and display a profile of activity not previously described for other anti-CD30 mAbs. In unmodified form, these antibodies, AC10 and HeFi-1 inhibit the growth of HD and the ALCL Line Karpas 299 and display in vivo antitumor activity in a tumor xenograft model of Hodgkin's disease.

9. Antitumor Activity of Chimeric AC10 in Subcutaneous L540CY Hodgkin's Disease Xenografts

9.1 Materials and Methods

Chimeric AC10 (cAC 10) was generated via homologous recombination essentially as previously described using human IgG1-kappa heavy and light chain conversion vectors (Yarnold and Fell, 1994, Cancer Res. 54: 506–512). These vectors were designed such that the murine immunoglobulin heavy and light chain constant region loci are excised and replaced by the human gamma 1 and kappa constant region loci via homologous recombination. The resulting chimeric hybridoma cell line expresses a chimeric antibody consisting of the heavy and light chain variable regions of the original monoclonal antibody and the human gamma 1 and kappa constant regions.

9.2 Results

To evaluate the efficacy of cAC10 in vivo, SCID mice were implanted subcutaneously with L540cy cells as described above. When the tumors reached an average size of greater than 150 mm^3 the mice were divided into groups that were either untreated or treated with 2 mg/kg cAC 10 twice per week for a total of five injections. The tumors in the untreated mice rapidly grew to an average size of greater than 600 mm^3 (FIG. 6). In contrast, the average tumor size in the animals treated with cAC 10 remained about the same size.

10. SPECIFIC EMBODIMENTS CITATION OF REFERENCES

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references, including patent applications, patents, and scientific publications, are cited herein, the disclosures of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 32

<210> SEQ ID NO 1
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(351)

<400> SEQUENCE: 1

```
cag atc cag ctg cag cag tct gga cct gag gtg gtg aag cct ggg gct       48
Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Val Val Lys Pro Gly Ala
 1               5                  10                  15

tca gtg aag ata tcc tgc aag gct tct ggc tac acc ttc act gac tac       96
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30

tat ata acc tgg gtg aag cag aag cct gga cag gga ctt gag tgg att      144
Tyr Ile Thr Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

gga tgg att tat cct gga agc ggt aat act aag tac aat gag aag ttc      192
Gly Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

aag ggc aag gcc aca ttg act gta gac aca tcc tcc agc aca gcc ttc      240
Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Phe
65                  70                  75                  80

atg cag ctc agc agc ctg aca tct gag gac act gct gtc tat ttc tgt      288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

gcg aac tat ggt aac tac tgg ttt gct tac tgg ggc caa ggg act cag      336
Ala Asn Tyr Gly Asn Tyr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Gln
            100                 105                 110

gtc act gtc tct gca                                                 351
Val Thr Val Ser Ala
        115
```

<210> SEQ ID NO 2
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 2

```
Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Val Val Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30

Tyr Ile Thr Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Phe
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Asn Tyr Gly Asn Tyr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Gln
            100                 105                 110

Val Thr Val Ser Ala
        115
```

-continued

<210> SEQ ID NO 3
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 3

gactactata taacc 15

<210> SEQ ID NO 4
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

Asp Tyr Tyr Ile Thr
1 5

<210> SEQ ID NO 5
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

tggatttatc ctggaagcgg taatactaag tacaatgaga agttcaaggg c 51

<210> SEQ ID NO 6
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 6

Trp Ile Tyr Pro Gly Ser Gly Asn Thr Lys Tyr Asn Glu Lys Phe Lys
1 5 10 15

Gly

<210> SEQ ID NO 7
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 7

tatggtaact actggtttgc ttac 24

<210> SEQ ID NO 8
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

Tyr Gly Asn Tyr Trp Phe Ala Tyr
1 5

<210> SEQ ID NO 9
<211> LENGTH: 333
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(333)

<400> SEQUENCE: 9

gac att gtg ctg acc caa tct cca gct tct ttg gct gtg tct cta ggg 48
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

-continued

```
cag agg gcc acc atc tcc tgc aag gcc agc caa agt gtt gat ttt gat      96
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Phe Asp
            20                  25                  30

ggt gat agt tat atg aac tgg tac caa cag aaa cca gga cag cca ccc     144
Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

aaa gtc ctc atc tat gct gca tcc aat cta gaa tct ggg atc cca gcc     192
Lys Val Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala
    50                  55                  60

agg ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc aac atc cat     240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80

cct gtg gag gag gag gat gct gca acc tat tac tgt cag caa agt aat     288
Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
                85                  90                  95

gag gat ccg tgg acg ttc ggt gga ggc acc aag ctg gaa atc aaa         333
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> SEQ ID NO 10
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

```
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Phe Asp
            20                  25                  30

Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Val Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
                85                  90                  95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> SEQ ID NO 11
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 11

```
aaggccagcc aaagtgttga ttttgatggt gatagttata tgaac                    45
```

<210> SEQ ID NO 12
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

```
Lys Ala Ser Gln Ser Val Asp Phe Asp Gly Asp Ser Tyr Met Asn
 1               5                  10                  15
```

<210> SEQ ID NO 13
<211> LENGTH: 21
<212> TYPE: DNA

-continued

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

gctgcatcca atctagaatc t 21

<210> SEQ ID NO 14
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

Ala Ala Ser Asn Leu Glu Ser
1 5

<210> SEQ ID NO 15
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

cagcaaagta atgaggatcc gtggacg 27

<210> SEQ ID NO 16
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

Gln Gln Ser Asn Glu Asp Pro Trp Thr
1 5

<210> SEQ ID NO 17
<211> LENGTH: 375
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(375)

<400> SEQUENCE: 17

```
gag gtg aag ctg gtg gag tct gga gga ggc ttg gta cag cct ggg ggt       48
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15

tct ctg aga ctc tcc tgt gca act tct ggg ttc acc ttc agt gat tac       96
Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30

tat atg aac tgg gtc cgc cag cct cca gga aag gct ctt gag tgg ttg      144
Tyr Met Asn Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
        35                  40                  45

ggt ttt att aga aac aaa gct aat ggt tac aca aca gag ttc agt gca      192
Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Phe Ser Ala
    50                  55                  60

tct gtg atg ggt cgg ttc acc atc tcc aga gat gat tcc caa agc atc      240
Ser Val Met Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65                  70                  75                  80

ctc tat ctt cag atg aac acc ctg aga gct gag gac agt gcc act tat      288
Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr
                85                  90                  95

tac tgt gca aga gat ccc ccc tat ggt aac ccc cat tat tat gct atg      336
Tyr Cys Ala Arg Asp Pro Pro Tyr Gly Asn Pro His Tyr Tyr Ala Met
            100                 105                 110

gac tac tgg ggt caa gga acc tca gtc acc gtc tcc tca                  375
Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
```

-continued

115 120 125

<210> SEQ ID NO 18
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 18

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Asn Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Phe Ser Ala
50 55 60

Ser Val Met Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65 70 75 80

Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr
85 90 95

Tyr Cys Ala Arg Asp Pro Pro Tyr Gly Asn Pro His Tyr Tyr Ala Met
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 19
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

gattactata tgaac 15

<210> SEQ ID NO 20
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

Asp Tyr Tyr Met Asn
1 5

<210> SEQ ID NO 21
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

tttattagaa acaaagctaa tggttacaca acagagttca gtgcatctgt gatgggt 57

<210> SEQ ID NO 22
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 22

Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Phe Ser Ala Ser
1 5 10 15

Val Met Gly

-continued

<210> SEQ ID NO 23
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 23

gatccccct atggtaaccc ccattattat gctatggact ac 42

<210> SEQ ID NO 24
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 24

Asp Pro Pro Tyr Gly Asn Pro His Tyr Tyr Ala Met Asp Tyr
1 5 10

<210> SEQ ID NO 25
<211> LENGTH: 333
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(333)

<400> SEQUENCE: 25

gac att gtg ctg acc cag tct cct gct tcc tta gct gtt tct ctg ggg 48
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

cag agg gcc acc atc tca tgc agg gcc agc aaa agt gtc agt gca tct 96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
20 25 30

ggc tat aat tat atg cac tgg tac caa cag aaa gca ggg cag cca ccc 144
Gly Tyr Asn Tyr Met His Trp Tyr Gln Gln Lys Ala Gly Gln Pro Pro
35 40 45

aaa ctc ctc atc cat ctt gca tcc aac cta gaa tct ggg gtc cct gcc 192
Lys Leu Leu Ile His Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

agg ttc agt ggc agt ggg tct ggg aca gac ttc acc ctc aac atc cat 240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

cct gtg gag gag gag gat gct tca acc tat tac tgt cag cac agt ggg 288
Pro Val Glu Glu Glu Asp Ala Ser Thr Tyr Tyr Cys Gln His Ser Gly
85 90 95

gag ctt cca ttc acg ttc ggc tcg ggg aca aag ttg gaa ata aaa 333
Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 26
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 26

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Ala Ser
20 25 30

Gly Tyr Asn Tyr Met His Trp Tyr Gln Gln Lys Ala Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile His Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

-continued

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ser Thr Tyr Tyr Cys Gln His Ser Gly
85 90 95

Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> SEQ ID NO 27
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 27

agggccagca aaagtgtcag tgcatctggc tataattata tgcac 45

<210> SEQ ID NO 28
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 28

Arg Ala Ser Lys Ser Val Ser Ala Ser Gly Tyr Asn Tyr Met His
1 5 10 15

<210> SEQ ID NO 29
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 29

cttgcatcca acctagaatc t 21

<210> SEQ ID NO 30
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 30

Leu Ala Ser Asn Leu Glu Ser
1 5

<210> SEQ ID NO 31
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 31

cagcacagtg gggagcttcc attcacg 27

<210> SEQ ID NO 32
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 32

Gln His Ser Gly Glu Leu Pro Phe Thr
1 5

What is claimed is:

1. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject, in an amount effective for said treatment, (a) an antibody that (i) immunospecifically binds CD30 and (ii) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, wherein said antibody exerts the cytostatic or cytotoxic effect on the Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent and in the absence of cells other than cells of said Hodgkin's Disease cell line; and (b) a pharmaceutically acceptable carrier.

2. The method of claim 1, wherein the antibody is human, humanized or chimeric.

3. The method of claim 1, further comprising administering chemotherapy to said subject.

4. The method of claim 1, wherein the antibody is conjugated to a cytotoxic agent.

5. The method of claim 1, wherein the antibody is a fusion protein comprising an antigen binding region that immunospecifically binds to CD30 and an amino acid sequence of a second protein that is not an antibody.

6. The method of claim 4 or 5, further comprising administering chemotherapy to said subject.

7. The method of claim 1, wherein the cytostatic or cytotoxic effect of the antibody is exhibited upon performing a method comprising:

(a) contacting a culture of the Hodgkin's Disease cell line with the antibody, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours;

(b) exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72 hour period; and

(c) measuring the incorporation of the ^{3}H-thymidine into cells of the culture,

wherein the antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the antibody.

8. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject an amount of an antibody, which antibody (a) competes for binding to CD30 with monoclonal antibody AC10 or HeFi-1, and (b) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line in the absence of cell other than cells of said Hodgkin's Disease cell line, which amount is effective for the treatment of Hodgkin's Disease.

9. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject an amount of an antibody, which antibody (a) comprises the amino acid sequence that has at last 95% identity to SEQ ID NO:2, (b) immunospecifically binds CD30, and (c) exerts a cytostatic or cytostatic effect on a Hodgkin's Disease cell line in the absence of cells other than cells of said Hodgkin's Disease cell line which amount is effective for the treatment of Hodgkin's Disease.

10. The method of any one of claims 8 or 9, wherein the antibody is a human, humanized or chimeric antibody.

11. The method of any one of claims 8 or 9, wherein comprising administering chemotherapy to said subject.

12. The method of any one of claims 8 or 9, wherein the antibody is conjugated to a cytotoxic agent.

13. The method of any one of claims 8 or 9, wherein the antibody is fusion protein comprising an antigen binding region that immunospecifically binds to CD30 and the amino acid sequence of a second protein.

14. The method of claim 13, further comprising administering chemotherapy to the subject.

15. The method of claim 14, further comprising administering chemotherapy to the subject.

16. The method of any one of claims 8 or 9, wherein the cytostatic or cytotoxic effect is exhibited upon performing a method comprising:

(a) contacting a culture of the Hodgkin's Disease cell line with the antibody, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours;

(b) exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72 hour 72 our period; and

(c) measuring the incorporation of the ^{3}H-thymidine into cells of the culture, wherein the antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the antibody.

17. A method for the treatment of Hodgkin's Disease in a subject comparing administering to the subject, in an amount effective for said treatment, (a) an antibody that (i) immunospecifically binds CD30 and (ii) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, wherein the antibody exerts the cytostatic or cytotoxic effect on the Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent and (b) a pharmaceutically acceptable carrier,

wherein the cytostatic or cytotoxic effect of the antibody is exhibited upon performing a method comprising:

(A) immobilizing said antibody in a well, said well having a culture area of about 0.33 cm^2;

(B) adding about 5,000 cells of the Hodgkin's Disease cell line in the presence of RPMI with 20% fetal bovine serum to the well:

(C) culturing the cells in the presence of said antibody and RPMI with 20% fetal bovine scrum for a period of 72 hours to form a Hodgkin's Disease cell culture;

(D) exposing the Hodgkin's Disease cell culture to 0.5 μCi/well of ^{3}H-thymidine during the final 8 hours of said 72 hour period; and

(E) measuring the incorporation of the ^{3}H-thymidine into cells of the Hodgkin's Disease cell culture,

wherein the antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the Hodgkin's Disease cell culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the antibody.

18. A method for the treatment of Hodgkin's Disease in a subject comprising administering to the subject, in an amount effective for said treatment, (a) a chimeric, humanized or human antibody that (i) immunospecifically binds CD30 and (ii) exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, wherein the chimeric, humanized or human antibody exerts the cytostatic or cytotoxic effect on Hodgkin's Disease cell line in the absence of conjugation to a cytostatic or cytotoxic agent and (b) a pharmaceutically acceptable carrier,

wherein the cytostatic or cytotoxic effect of the chimeric, humanized or antibody is exhibited upon performing a method comprising:

(A) contacting a culture of the Hodgkin's Disease cell line with the chimeric, humanized or human antibody, said culture being of about 5,000 cells in a culture area of about 0.33 cm^2, said contacting being for a period of 72 hours;

(B) adding a cross-linking antibody to the Hodgkin's Disease cell line, the cross-linking antibody binding to the chimeric, humanized or human antibody;

(C) exposing the culture to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of said 72-hour period; and

(D) measuring the incorporation of the ^{3}H-thymidine into cells of the culture, wherein the chimeric, humanized or human antibody has a cytostatic or cytotoxic effect on the Hodgkin's Disease cell line if the cells of the culture have reduced ^{3}H-thymidine incorporation compared to cells of the same Hodgkin's Disease cell line cultured under the same conditions but not contacted with the chimeric, humanized or human antibody.

* * * * *

Return To:

USPTO Home Page

Finance Online Shopping Page

United States Patent and Trademark Office

Maintenance Fee Statement

10/05/2011 07:34 PM EDT

Patent Number: 7090843

Customer Number: 000000000

SEATTLE GENETICS, INC.
21823 30TH DRIVE SE
BOTHELL WA 98021

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR-CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
7,090,843	$980.00	$0.00	02/16/10	09/724,406	08/15/06	11/28/00	04	NO	SEGEN 0030-00101US

Click here to obtain your Maintenance Fee Statement as a PDF.
Please note that in order to view your Maintenance Fee Statement as a PDF, you must have Adobe's Acrobat Reader installed.
If you do not have it, download it for FREE!

Get Acrobat Reader

Return To:

USPTO Home Page

Finance Online Shopping Page

United States Patent and Trademark Office

Patent Bibliographic Data				09/28/2011 02:52 PM	
Patent Number:	7090843		**Application Number:**	09724406	
Issue Date:	08/15/2006		**Filing Date:**	11/28/2000	
Title:	RECOMBINANT ANTI-CD30 ANTIBODIES AND USES THEREOF				
Status:	8th year fee window opens: 08/15/2013			**Entity:**	Large
Window Opens:	08/15/2013	**Surcharge Date:**	02/19/2014	**Expiration:**	N/A
Fee Amt Due:	Window not open	**Surchg Amt Due:**	Window not open	**Total Amt Due:**	Window not open
Fee Code:	1552	MAINTENANCE FEE DUE AT 7.5 YEARS			
Surcharge Fee Code:					
Most recent events (up to 7):	02/16/2010 01/06/2010	Payment of Maintenance Fee, 4th Year, Large Entity. Pat Hldr no Longer Claims Small Ent Stat --- End of Maintenance History ---			
Address for fee purposes:	SEATTLE GENETICS, INC. 21823 30TH DRIVE SE BOTHELL WA 98021				

Run Another Query

7

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 71,634

Seattle Genetics, Inc.
21823 30th Drive, SE
Bothell, WA 98021

Attention: Bruce Hart, Ph.D., RAC
Director, Regulatory Affairs

Dear Dr. Hart:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned:	71,634
Sponsor:	Seattle Genetics
Name of Drug:	SGN-35 (cAC10-vcMMAE (4))
Date of Submission:	June 27, 2006
Date of Receipt:	June 28, 2006

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before July 28, 2006, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports [21 CFR 312.33].

As required by the Food and Drug Modernization Act and the Best Pharmaceuticals for Children Act, you are also responsible for registering certain clinical trials involving your drug product in the Clinical Trials Data Bank (http://clinicaltrials.gov & http://prsinfo.clinicaltrials.gov/). If your drug is intended for the treatment of a serious or life-threatening disease or condition and you are conducting clinical trials to test its effectiveness, then you must register these trials in the Data Bank. Although not required, we encourage you to register effectiveness trials for non-serious diseases or conditions as well as non-effectiveness trials for all diseases or conditions, whether or not they are serious or life-threatening. Additional information on registering your clinical trials, including the required and optional data elements and the FDA Draft Guidance for Industry, "Information Program on Clinical Trials for Serious or Life-Threatening Diseases and Conditions," is available at the Protocol Registration System (PRS) Information Site http://prsinfo.clinicaltrials.gov/.

Please cite the IND number listed above at the top of the first page of any communications concerning this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Oncology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Christy Cottrell, Consumer Safety Officer, at (301) 796-1347.

Sincerely,

{See appended electronic signature page}

Dotti Pease
Chief, Project Management Staff
Division of Drug Oncology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Christy Cottrell
7/3/2006 11:07:38 AM
Signing for Dotti Pease

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL 125388/0

BLA ACKNOWLEDGEMENT
March 2, 2011

Seattle Genetics, Inc.
Attention: Elaine Waller
Senior Vice President, Regulatory Affairs
21823 30th Drive Southeast
Bothell, WA 98021

Dear Ms. Waller:

We have received your Biologics License Application (BLA) submitted under section 351 of the Public Health Service Act (PHS Act) for the following:

Name of Biological Product: brentuximab vedotin

Date of Application: February 25, 2011

Date of Receipt: February 28, 2011

Our Submission Tracking Number (STN): BL 125388/0

Proposed Use: Relapsed or refractory Hodgkin's Lymphoma

If you have not already done so, promptly submit the content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format as described at http://www.fda.gov/oc/datacouncil/spl.html. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action. The content of labeling must conform to the format and content requirements of revised 21 CFR 201.56-57.

You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) [42 USC §§ 282 (i) and (j)], which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No, 110-85, 121 Stat. 904).

Title VIII of FDAAA amended the PHS Act by adding new section 402(j) [42 USC § 282(j)], which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices.

In addition to the registration and reporting requirements described above, FDAAA requires that, at the time of submission of an application under section 505 of the FDCA, the application must be accompanied by a certification that all applicable requirements of 42 USC § 282(j) have been met. Where available, the certification must include the appropriate National Clinical Trial (NCT) numbers [42 USC § 282(j)(5)(B)].

You did not include such certification when you submitted this application. You may use Form FDA 3674, "Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank," [42 U.S.C. § 282(j)] to comply with the certification requirement. The form may be found at http://www.fda.gov/opacom/morechoices/fdaforms/default.html.

In completing Form FDA 3674, you should review 42 USC § 282(j) to determine whether the requirements of FDAAA apply to any clinical trial(s) referenced in this application. Please note that FDA published a guidance in January 2009, "Certifications To Accompany Drug, Biological Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of the Food and Drug Administration Amendments Act of 2007," that describes the Agency's current thinking regarding the types of applications and submissions that sponsors, industry, researchers, and investigators submit to the Agency and accompanying certifications. Additional information regarding the certification form is available at: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FoodandDrugAdministrationAmendmentsActof2007/ucm095442.htm. Additional information regarding Title VIII of FDAAA is available at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-014.html. Additional information for registering your clinical trials is available at the Protocol Registration System website http://prsinfo.clinicaltrials.gov/.

When submitting the certification for this application, **do not** include the certification with other submissions to the application. Submit the certification within 30 days of the date of this letter. In the cover letter of the certification submission clearly identify that it pertains **BLA 125388/0** submitted on February 25, 2011, and that it contains the FDA Form 3674 that was to accompany that application.

If you have already submitted the certification for this application, please disregard the above.

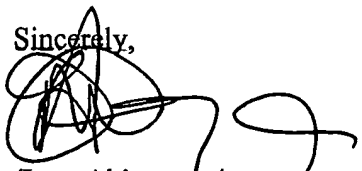
The BLA Submission Tracking Number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an unnecessary delay in processing which could have an adverse impact on the review of the submission.

If you have any questions, call me at (301) 796-9634.

Sincerely,



/Lara Akinsanya/
Lara Akinsanya, M.S.
Regulatory Health Project Manager
Division of Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research